

## PHYSIOLOGICAL EVIDENCE FOR THE OCCURRENCE OF PATHWAYS SHUNTING BLOOD AWAY FROM THE SECONDARY LAMELLAE OF EEL GILLS

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### SUMMARY

1. Several cardiovascular and respiratory measurements have been performed in eels before and after intravenous injections of adrenaline. These experiments have allowed a comparison to be made of values for the cardiac output determined directly ( $\dot{Q}$ ) and using the Fick principle ( $\dot{Q}_F$ ) on individual fish under these two conditions.

2. Under control conditions it was shown that  $\dot{Q}_F/\dot{Q} = 0.72$ , indicating that about 30% of the mixed venous blood afferent to the gills is returned directly to the heart and bypasses the lamellar circulation via veno-venous anastomoses between the afferent filament arteries and the central venous space of the gill filaments.

3. Adrenaline, which during winter only has its action due to stimulation of  $\alpha$ -adrenoreceptors, induced a hypoventilation but no changes in cardiac output in spite of a bradycardia. The oxygen content of the mixed venous blood was markedly increased whereas  $C_{a,O_2}$  remained unchanged as did the percentage utilization of oxygen from the water as it passed over the gills. The efferent blood flow from the gills after injection of adrenaline was almost equal to the total cardiac output.

It is suggested that such a circulatory change was due to adrenaline-mediated constriction of veno-venous anastomoses in the gills of the eel.

### INTRODUCTION

Blood pathways through fish gills have classically been thought to consist of a direct communication between afferent and efferent vessels through a complex network of blood channels in the secondary lamellae where gas exchanges take place. Thus the whole cardiac output entering the ventral aorta would pass along this respiratory pathway before being collected in the dorsal aorta and thence to the tissues. When considered in detail, this single circulation is bound to have further complications because of the fate of blood supplying the gill tissues themselves, but the volume involved is probably extremely small.

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Although some earlier studies (Müller, 1839; Reiss, 1881) indicated a greater complexity to the circulation than was usually taught in comparative anatomy, nevertheless, it is only during the past 15 years that the possibility of other blood pathways being involved has received detailed attention. Studies of Steen & Krusysse (1964) opened up this question from both a morphological and physiological point of view. In order to interpret an increase in arterial  $P_{O_2}$  following the injection of adrenaline they suggested that this drug caused dilation of respiratory pathways and constriction of another non-respiratory pathway consisting of the central compartment which communicated with both the afferent and efferent filament arteries. Their 'filament shunt model' supposed that  $P_{O_2}$  of dorsal aorta blood could be raised by increasing the proportion of blood flowing through the secondary lamellar pathway. This model was supported by Richards & Fromm (1969), but a number of observers failed to observe the passage of India ink injected into the ventral aorta unless the pressure levels were especially high (Byckowska-Smyk, Morgan, Munshi in Hughes, 1972). Other studies (Cameron, 1974; Morgan & Tovell, 1973) also shed some doubt on the presence of such shunt pathways and it is also improbable hydrodynamically because the pressure in both the afferent and efferent filament arteries must exceed that in the central compartment (Hughes, 1978). An alternative way of explaining the increased arterial oxygen level involving recruitment of secondary lamellar pathways has been suggested (Hughes, 1972) and received support from the observations of Davis (1972). It is quite probable that both shunting of blood and recruitment of secondary lamellae are components of the mechanism which controls the extent and distribution of blood exposed to the water (Hughes, 1976; Booth, 1978).

Most recent anatomical and physiological studies have shown that variations occur between different species with respect to the presence and numbers of anastomoses between the afferent filament artery and the central venous space compared with those occurring between the central compartment and the efferent filament arteries (Vogel *et al.* 1973, 1974, 1976, 1978; Holbert *et al.* 1979; Laurent & Dunel, 1976). In the eel, the main species studied by Steen & Krusysse, there are about three times more anastomoses of the first type relative to those along the efferent filament artery (W. Vogel, unpublished). Other studies on the eel circulation have confirmed the presence of two such pathways through the gill filament and show the presence of sphincters on the efferent arterioles leading from the secondary lamellar circulation. Dilation of such sphincters would open the lamellar pathways and so decrease the flow of blood from the central filament space into the branchial vein and thence to the heart. Such an increase in blood flow through the secondary lamellar pathway is enhanced by adrenaline whilst acetylcholine increases flow via the venous pathway by constricting the efferent arteriolar sphincter (Dunel & Laurent, 1977). These views are in general agreement with studies based on whole animal preparations (Peyraud-Waitzenegger, 1979).

Thus it has become a common feature of many hypotheses that a proportion of the blood entering the gills may return directly to the heart without oxygenation, and that the proportion of this flow may be varied in relation to the respiratory environment. There is, however, no physiological evidence to indicate the relative sizes

These portions of the cardiac output and whether they can vary. The main purpose of the present study was to attempt such an estimation by measuring cardiac output directly in the ventral aorta, and also, by means of the Fick principle, the flow of blood passing across the gills, any difference between these values being considered to represent the portion of the cardiac output which is returned directly to the heart in the branchial vein from the afferent filament arteries. The eel is an ideal fish for such experiments because of the elongated nature, and ready accessibility, of its ventral aorta. A preliminary account of this work has already been published (Hughes, Peyraud, Peyraud-Waitzenegger & Soulier, 1981).

#### MATERIALS AND METHODS

Eels (600–1000 g) were obtained from commercial dealers and kept in aquaria at the laboratory in running tap water for several weeks before the experiments which took place in winter at a temperature of  $9.5 \pm 1$  °C. The operative procedures were carried out in the following sequence, in all cases the specimens being anaesthetized in a 3% solution of urethane.

(1) The spinal cord was severed between the medulla and spinal cord; the body surface was stitched. On recovery, the animal made few active swimming movements.

(2) A polyvinyl catheter was inserted into the pneumogastric artery and the intestinal vein, the former to obtain samples of arterial blood and to measure dorsal aortic pressure, the latter for injections (physiological saline, adrenaline, etc.). A mid-ventral incision in the branchial region was made to expose the ventral aorta which is relatively elongated in this species. Clamps were placed on the aorta and the arterial end of the bulbous arteriosus; the ventral aorta severed, and a length of polyvinyl tubing 5–6 cms long (mean inside diameter 1.5 mm, but depends on size of fish) was inserted into the ventral aorta, and a similar piece into the bulbous arteriosus. These two portions were joined to form an extracorporeal circulation following release of the clamps. The connective tissue and skin were stitched up. The eels survived this operation extremely well.

To reduce blood coagulation, heparin was injected intravenously (500 IU/kg) each day.

(3) The fish was allowed to recover overnight and the next day tubes were stitched and glued (Histacryl) into the round opercular openings in order that all the expired water could be collected. These tubes were relatively wide (1 cm diameter) and were joined by a Y-piece in which a small flap of rubber formed a valve which mimicked the action of the passive opercular valves and so prevented the reflux of water into the opercular cavities. The expired water was led away and collected in a vessel, the level of which was carefully adjusted to be identical to that of the chamber containing the fish so that no hydrostatic pressure gradient was present to affect ventilation. ECG leads were implanted on both sides of the heart together with leads inserted in the opercula which made it possible to record the ventilatory frequency as changes in impedance between these electrodes (Peyraud & Ferret-Bouin, 1960). The fish were allowed to recover from the surgical procedures for at least 24 h prior to experimentation. Measurements made using these preparations were as follows:

(1) Cardiac output: the external ventral aorta circulation through the polyvinyl cannula of 1.5 mm diameter made it possible to measure directly the cardiac output which was proportional to the blood flow velocity determined using a Doppler velocimeter (Sonicaid). Calibration was carried out using a pulsatile Filamatic pump over the same range of frequencies and flow rates which are normal for the eel circulation. This particular method for determining cardiac output was used following attempts using several other methods and it was found to have the great advantage that calibration was possible using conditions identical to those which occurred during the experiments. A digital read-out of velocity was obtained in units which could be converted to cardiac output.

(2) In order to determine the cardiac output using the Fick method, it was essential to make good measurements of  $C_{a,O_2}$  and  $C_{v,O_2}$ . The method finally used was developed by modification of the Lex-O<sub>2</sub>-Con analyser (Hughes, Belaud, Peyraud & Adcock, 1982) to give more consistent results for the low values found in fish blood. Values for oxygen content of mixed venous blood ( $C_{r,O_2}$ ) were obtained by sampling from the external ventral aorta circulation.

(3) As described above, a direct method for the determination of ventilation volume was utilized and this not only gives good values for ventilation volume, but also enables measurements to be made of the  $P_{O_2}$  in mixed expired water and avoids sampling problems inherent in indirect methods. Ventilation volume was registered by means of a self-emptying siphon the frequency of which was recorded on the chart recorder (Hughes & Ballintijn, 1968).

(4)  $P_{O_2}$  in the inspired and expired water was determined in samples which were passed over cuvetted Radiometer electrodes maintained at the appropriate temperature by water from the experimental circulation. A pump ensured a constant velocity of flow over the electrode.

During an experiment, simultaneous recordings were made from the velocimeter, blood pressure in the dorsal and ventral aorta, the ECG, the ventilation volume and opercular frequency.

#### CALCULATIONS

The diagram given in Fig. 1 indicates the basic blood circulation and the measurements that have been made. The O<sub>2</sub> uptake ( $\dot{V}_{O_2}$ ) was calculated from the conventional relationship using ventilatory water flow ( $\dot{V}_G$ ) and the inspired-expired  $P_{O_2}$  difference:

$$\dot{V}_{O_2}, \text{ gills} = \dot{V}_G(P_{\text{insp}} - P_{\text{exp}})\alpha_{w, O_2}.$$

The cardiac output ( $Q_F$ ) was estimated from the O<sub>2</sub> content difference between arterial and mixed venous blood ( $C_{a,O_2} - C_{v,O_2}$ ) and the oxygen uptake at the gills, according to the Fick principle:

$$Q_F = \frac{\dot{V}_{O_2}, \text{ gills}}{C_{a, O_2} - C_{v, O_2}}.$$

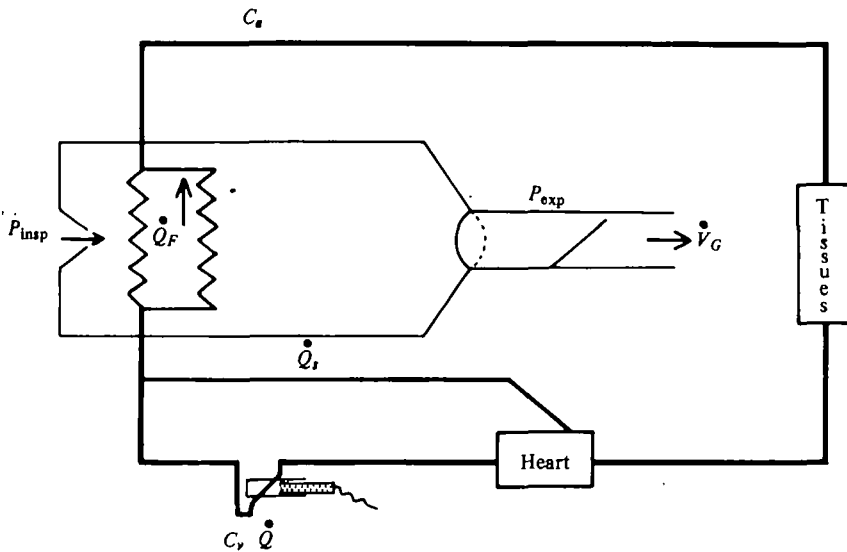


Fig. 1. Diagram to show the main features of the circulation of an eel together with symbols to indicate the measurements made in these experiments. The path of the water flow across the gills and symbols adopted are also indicated.

RESULTS

In order to assess the influence of the complex operative procedures a comparison was made of the heart rate and dorsal arterial blood pressure of 5 eels (a) initially fitted with only the pre-cardiac electrodes and a catheter in the coeliac artery and (b) the same specimens when the whole operation had been completed. Thus in the latter case the fish had more extensive surgery in which the ventral aortic circulation was exteriorized and tubes were attached for collection of expired water from the opercular cavities. Results of this comparison are given in Table 1 and indicate that there is a slight (10%) but significant ( $P < 0.05$ ) decrease in the blood pressure

Table 1. Comparison of heart rate and dorsal arterial pressure of five eels following two different degrees of surgery: (1) control: eels fitted with ECG electrodes and catheter in coeliac artery to measure dorsal aortic pressure, (2) experimental: same eels in which (a) the ventral aorta has been exteriorized and (b) opercular collecting ducts have been added as described in text

	Heart rate (/min)					Dorsal arterial pressure (mmHg)				
Control	36	36	25	26	40	40	35	30	33	37
Experimental	42	38	26	37	40	38	32	25	31	34
Mean difference	$4 \pm 2.0248$					$-3 \pm 0.5477$				
<i>t</i>	1.975					5.47				
	NS, $P < 0.05$ .					Significantly different, $P < 0.05$ .				

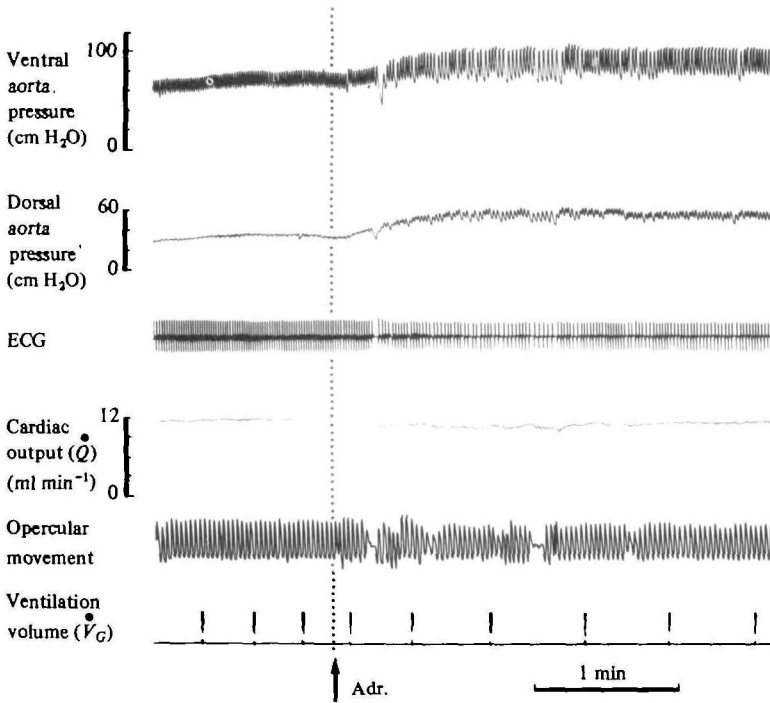


Fig. 2. Six-channel recording from an eel during an experiment in which adrenaline was injected into the coeliac artery. The rise in pressure in both the ventral and dorsal aorta is shown together with differences in pulse pressure. These are associated with a bradycardia but only a very slight fall in the cardiac output was measured using the velocimeter. The opercular movements also show a slight reduction in frequency which is associated with a fall in ventilation volume ( $\dot{V}_G$ ) as indicated by the reduction in frequency with which the self-filling siphon overflows.

recorded from the dorsal aorta following the more extensive operations. Heart rate was not significantly higher in these fish as compared with rates when they were less extensively operated. Moreover, it had previously been observed that, following a recovery period of 24 h, the insertion of a chronic catheter in the coeliac trunk did not increase the heart rate more than 10% of its control value (Peyraud-Waitzenegger, 1978). Some of the cardiovascular and ventilatory effects induced by the injection of adrenaline are illustrated by recordings shown in Fig. 2. The changes produced by the injection of adrenaline are also similar to those obtained in previous studies carried out during winter when the dorsal arterial aortic pressure, ECG and opercular movements had been recorded simultaneously in eels (Peyraud-Waitzenegger, Barthélemy & Peyraud, 1980). It can be seen that the blood pressure recorded from both the ventral and dorsal aorta is increased. There is a slight, but non-significant, decrease in cardiac output which is associated with a bradycardia. Such a result is not surprising as eels do not show an increase in cardiac output following injection of adrenaline during winter although this does occur in summer. The hypoventilation leads to a significant decrease in ventilatory water flow ( $\dot{V}_G$ ).

Table 2. *Anguilla anguilla*, mean values ( $\pm$  S.E.) for cardiac output, directly determined ( $\dot{Q}$ ), and by application of the Fick principle ( $\dot{Q}_F$ ) to measurements made on ten fish; changes following injection of adrenaline are also given

	Control	+ Adrenaline (2 $\mu$ g kg <sup>-1</sup> )
$\dot{V}_O$ (ml min <sup>-1</sup> kg <sup>-1</sup> )	57.58 $\pm$ 9.06	40.83 $\pm$ 7.14
$P_{\text{insp}} - P_{\text{exp}}$ (mmHg)	86.40 $\pm$ 10.30	81.80 $\pm$ 7.40
$\dot{V}_{O_2}$ , gills (ml h <sup>-1</sup> kg <sup>-1</sup> )	13.62 $\pm$ 1.51	9.01 $\pm$ 0.73
$C_{a, O_2}$ (mmol l <sup>-1</sup> ) (vols %)	2.17 $\pm$ 0.26 (4.87)	2.16 $\pm$ 0.30 (4.84)
$C_{v, O_2}$ (mmol l <sup>-1</sup> ) (vols %)	1.02 $\pm$ 0.21 (2.29)	1.45 $\pm$ 0.26 (3.25)
$\dot{Q}_F$ (ml min <sup>-1</sup> kg <sup>-1</sup> )	8.73 $\pm$ 1.16	10.93 $\pm$ 1.74
$\dot{Q}$ (ml min <sup>-1</sup> kg <sup>-1</sup> )	12.16 $\pm$ 1.71	11.68 $\pm$ 2.03
$\dot{Q}_F/\dot{Q}$	0.72 $\pm$ 0.02	0.94 $\pm$ 0.02

Table 3. Cardiac output of ten individual eels measured under control conditions and following injection of adrenaline (2  $\mu$ g/kg): determinations were made directly ( $\dot{Q}$ ) and by means of the Fick method ( $\dot{Q}_F$ ) in each case

Eel no.	$\dot{Q}_F$		$\dot{Q}$	
	Control	+ Adrenaline	Control	+ Adrenaline
1	6.25	8.92	10.50	9.52
2	12.54	17.85	19.20	19.82
3	10.07	12.37	13.22	13.38
4	16.73	22.03	23.05	25.10
5	7.76	6.65	8.45	6.55
6	5.96	6.38	6.60	6.45
7	5.53	6.85	7.97	7.59
8	6.12	7.98	7.88	7.96
9	10.12	14.08	14.60	13.97
10	6.25	6.26	10.15	6.49
Mean $\pm$ S.E.	8.73 $\pm$ 1.16	10.93 $\pm$ 1.74	12.16 $\pm$ 1.71	11.68 $\pm$ 2.03

$$|t| \dot{Q}_F = 3.21, P < 0.02.$$

$$|t| \dot{Q} = 0.99, \text{ not significant.}$$

A summary of results obtained in 10 experiments is given in Tables 2 and 3 and allows a comparison to be made between  $\dot{Q}$  and  $\dot{Q}_F$ . It appears that  $\dot{Q}_F$  is significantly less than  $\dot{Q}$  under the control conditions (ratio  $\dot{Q}_F/\dot{Q} = 0.72$ ). Following the injection of adrenaline there is a significant ( $P < 0.02$ , paired 't' test) increase of  $\dot{Q}_F$  so that the ratio  $\dot{Q}_F/\dot{Q}$  comes to be almost 1.

## DISCUSSION

### 1. Control values

The fish were resting and showed little movement during the experiments, mainly, of course, because of their spinal condition. Results reported in Table 1 suggest that, in spite of the complex operative procedures involved in these experiments the animals are in a physiological condition which is close to that of a normal eel. This is confirmed by the general appearance of the fish which survived the operation for ~~over~~ a week and the results reported have usually been completed within two or

three days. Ventilatory flow ( $\dot{V}_G$ ) varied according to the pattern of ventilation. Accordingly, for the data summarized in Table 2,  $\dot{V}_G$  and other values were only measured in eels which were exhibiting uninterrupted ventilation in order to obtain valid data for comparative estimation of the ventilatory effects of adrenaline. Measurements of ventilation volume were comparable to those obtained by van Dam (1938) in his classical studies of eel ventilation, and of Belaud *et al.* (1977).

The direct measurements of cardiac output ( $\dot{Q}$ ) under the present experimental conditions are considered to give a good indication of the cardiac output of resting intact eels. The values obtained are somewhat lower than those which have been measured in a number of other fish species (Satchell, 1971; Wood & Shelton, 1980). In this connection, it is important to remember that few of these studies have involved the direct measurement of cardiac output. Some measurements reported in the literature (Wood & Shelton, 1980; Jones *et al.* 1974; Farrel, 1978) have used types of flowmeter which are difficult to apply to these conditions and especially to calibrate. Thus electromagnetic flowmeters (Biotronex) were employed in the early stages of this work but were discontinued as it was not found possible to obtain a consistently snug fit around the ventral aorta and to ensure that the probe remained in a constant position throughout the experimental procedures as well as in a live fish for use in further calibration.

To overcome these problems it was necessary to set-up an external ventral aorta circulation in order to maintain a constant relative position between the vessel and transducer.

Few measurements have been made of cardiac output in eels. Using a thermal flow probe, Davie & Forster (1980) obtained values of  $11.4 \text{ ml. min}^{-1} \cdot \text{kg}^{-1}$  for *Anguilla australis* at  $17^\circ\text{C}$ . These values are very close to those obtained in the present experiments ( $12 \text{ ml. min}^{-1} \cdot \text{kg}^{-1}$ ) in *Anguilla anguilla* at  $9.5^\circ\text{C}$  with a Doppler flowmeter, by placing the transducer near the ventral aorta prosthesis. For the same species, using a dye dilution technique, Belaud (1977) obtained values of 16, 15 and  $20 \text{ ml. min}^{-1} \cdot \text{kg}^{-1}$  for 3 specimens at  $17^\circ\text{C}$ . Motais *et al.* (1969) obtained values of  $10\text{--}16.5 \text{ ml. min}^{-1} \cdot \text{kg}^{-1}$  for *A. anguilla* at  $19^\circ\text{C}$  using a Fick method following injection of saline containing tritiated water. These indirect methods show values somewhat higher than  $\dot{Q}_F$  obtained in the present experiments.

The Fick method has often been used in determination of the ventilation volume of fish but values obtained could not be regarded with complete confidence because of the difficulty in obtaining samples from the opercular cavity that are sufficiently representative of all the expired water (Hughes & Knights, 1968; Dejours, 1973). Nevertheless, this method was reliable under the conditions employed in the present work because the oxygen uptake was obtained using values for the  $P_{O_2}$  of mixed expired water ( $P_{\text{exp}}$ ), all of which was being collected for determining ventilation volume ( $\dot{V}_G$ ). Consequently, there are no complications due to any changes which might occur in the amount of oxygen uptake at the skin (Berg & Steen, 1965; Nonnotte & Kirsch, 1978). In the calculation of  $\dot{Q}_F$  the small quantity of oxygen entering the blood through the lining of the buccal cavity has been neglected. The measurement of the arterio-venous difference in  $C_{O_2}$  was only possible because of refinements to the Lex- $O_2$ -Con apparatus.



From the discussion so far, it would appear that both direct measurements of cardiac output ( $\dot{Q}$ ) and values obtained by the standard Fick method ( $\dot{Q}_F$ ) are reliable results and it is, therefore, of interest to enquire why  $\dot{Q}$  is different from  $\dot{Q}_F$ . From what is known of the anatomy of blood circulatory pathways in eel gills (Dunel & Laurent, 1980; Hughes, 1980) it seems reasonable to suppose that a portion of the afferent blood flow representing the observed difference ( $\dot{Q} - \dot{Q}_F$ ) is being shunted away from the efferent gill circulation by means of anastomoses between the afferent filament artery and the central filament space and thence to the heart by way of the venous circulation.

The present results appear to be the first demonstration using physiological methods for the presence of a circulatory shunt through the gills such as that which has been described anatomically. The finding that the shunted blood flow may be as much as 28% of the cardiac output strongly suggests that such a mechanism must have a significant function in the normal life of the eel. However, it must also be remembered that several, as yet undefined, aspects of gill function could influence this result, e.g. if the gill tissue consumes significant amounts of oxygen extracted directly from the water.

## 2. Effects of Adrenaline

The results reported in Table 2 lead to the conclusion that the changes produced by adrenaline in the gill circulation result in the proportion of the cardiac output passing to the efferent branchial circulation becoming greater than under control conditions. Such a change in gill circulation might be interpreted as an  $\alpha$ -mediated closure of anastomoses between afferent filament arteries and the central filament space.

Nevertheless, a complicating factor in the calculation of  $\dot{Q}_F$  is the lack of values for the amount of oxygen consumed by the gill tissues themselves as this would account for some change in the A/V difference used for the Fick calculations. The similarity of values for  $\dot{Q}$  and  $\dot{Q}_F$  after adrenaline suggests that this cannot be such a large proportion of the total  $\dot{V}_{O_2}$ .

Decrease in ventilation volume following the injection of adrenaline involving a reduction in both amplitude and frequency has been reported (Peyraud-Waitzenegger, 1980) as a typical ventilatory effect of adrenaline during winter. Associated with this hypoventilation, there is a decrease in oxygen uptake but it was surprising that the difference in oxygen tension between the inspired and expired water remained unchanged.

Two factors to be considered in the interpretation of this observation are:

(1) The absence of any change in utilization of  $O_2$  may be regarded as a consequence of an adrenaline-induced increase of  $C_{v,O_2}$  with a consequent decrease in the water/blood  $O_2$  diffusion difference. Such a rise in the oxygen content of venous blood may be partly due to a reduction in oxygen uptake at the tissues possibly due to an adrenaline-induced systemic vasoconstriction. The rise in  $C_{v,O_2}$  would lead to a reduction in the mean difference in oxygen tension between the water and blood and although effectiveness of oxygenation of the blood would appear to remain about the same, it is possible that there is a reduction in the capacity rate ratio ( $\dot{V}_{O_2}, \alpha_{w,O_2}$ )/

( $Q_F \cdot \alpha_b \cdot O_2$ ) compensated by an increase in the number of transfer units (Hughes & Shelton, 1962), i.e. an increase in effective surface area or a decrease in diffusion distances for  $O_2$  transfer.

(2) The increase of efferent blood flow from the gills does not imply that the portion of afferent blood which is then not by-passed because of the closure of veno-venous anastomoses will necessarily circulate through lamellar respiratory pathways. In this connexion it should be appreciated that not all pathways through the secondary lamellae are equally perfused under all conditions. Several observers have noted (Chan, 1969; Hughes, 1976, 1979; Rankin, 1976) especially in eels, that under certain conditions blood flow may be restricted to the marginal channels whereas at other times more blood may flow through the proximal channels where diffusion distances for gas exchange between the water and blood would be extremely large; in fact, this would constitute an intralamellar shunt. Nevertheless, all blood passing through these different secondary lamellar pathways would become mixed and enter the efferent filament and efferent branchial arteries which collect in the dorsal aorta. Furthermore, it should not be forgotten that present experiments were carried out in winter when only  $\alpha$ -adrenoceptive reactivity has been observed in eels (Peyraud-Waitzenegger, 1979; Peyraud-Waitzenegger *et al.* 1980).

Thus it might be supposed that in the present experiments adrenaline failed to produce vasodilation of sphincters on the lamellar arterioles which would have opened unused respiratory lamellar pathways as has been proposed to account for the catecholamine-induced  $P_{a,O_2}$  increase observed in summer. Consequently the surplus of cardiac output flowing from afferent to efferent filament arteries resulting from the  $\alpha$ -mediated closure of veno-venous anastomoses may have become directed through some of the non-respiratory intralamellar pathways discussed above.

Results obtained following injection of adrenaline indicating a reduction in shunt flow should be borne in mind when interpreting experiments (e.g. Cameron, 1974) purporting to show an absence of shunts if the conditions under which they were carried out might have increased the levels of blood catecholamines and hence reduced or closed the shunt pathways.

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