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Gill Function in an Elasmobranch

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Summary. Highly efficient oxygen uptake in elasmobranchs, as indicated by frequent excess of P_{aO_2} over P_{EO_2} has previously been ascribed to the operation of multicapillary rather than counter-current gas exchange by the gills. Analysis of models shows that, at maximum efficiency, a multicapillary system cannot account for values of P_{aO_2} greater than $(P_{IO_2} + P_{EO_2})/2$. In Port Jackson sharks *Heterodontus portusjacksoni* P_{aO_2} commonly exceeds $(P_{IO_2} + P_{EO_2})/2$, which indicates the operation of a functional counter-current at the respiratory surface. The anatomical basis of this counter-current is provided by the demonstration that a continuous flow of water passes between the secondary lamellae into septal canals and thence via the parabronchial cavities to the exterior.

Introduction

In teleost fish, the efficiency of gas exchange at the gills is enhanced by a counter-current flow of water and blood at the respiratory surface (Hughes and Shelton, 1962; Hughes, 1963; Steen and Kruyse, 1964). In elasmobranchs, on the other hand, Hughes and Shelton (1962) and Piiper and Schumann (1967) have suggested that the presence of large interbranchial septa prevents the operation of a similar counter-current. However, Piiper and Schumann (1967) found oxygen uptake by a dogfish *Scyliorhinus stellaris* to be more efficient than a simple concurrent system would allow. On anatomical grounds they rejected the possibility that this enhanced efficiency was the result of a counter-current system; "... respiratory water cannot easily continue on its way (between the secondary lamellae) but is obstructed by the interbranchial septum. Therefore the expired water may have to flow along the length of the gill filaments attached to the septal wall on the greater part of their length".

To explain their data they proposed that the morphology of elasmobranch gills was consistent with a "multicapillary" model. This is similar to the model proposed by Zeuthen (1942) for gas exchange in birds, and to the "multivillous stream" proposed by Metcalfe, Bartels and Moll (1967) in their discussion of placental gas exchange. In a later paper however, Piiper and Baumgarten-Schumann (1968) based their analysis of the effectiveness of gas exchange in *Scyliorhinus stellaris* on a counter-current system so these authors cannot be considered as decided antagonists of counter-current or protagonists of multicapillary theories. Kempton (1969), in an anatomical study of the gills of *Squalus acanthias* commented that whereas the morphology of its gills was consistent with a multicapillary exchange system more information was needed about the details of water flow before a definite conclusion could be reached. Kempton pointed out that if water moved "between adjacent secondary lamellae to the enlarged water channel . . ." then a countercurrent would occur between blood and water. Woskoboynikoff (1932) had in fact suggested that the water flowed in this manner in elasmobranchs; however, Kempton considered it doubtful that water would follow this route and hence he subscribed to the multicapillary theory. Grigg (1970), studying the path followed by water in traversing the gills of the Port Jackson shark, has shown that the water flows between the secondary lamellae and into the septal canals so that conditions exist for operation of a counter-current system. This does not, by itself, prove that such a system does operate and the question will now be pursued further.

Initially one can ascertain whether blood leaving the gills has a higher P_{O_2} or a lower P_{CO_2} than the expired respiratory medium. If it does, exchange of oxygen and carbon dioxide cannot be accounted for by a simple concurrent system. Lenfant and Johansen (1966) found no such evidence in *Squalus suckleyi*, although with improved experimental technique negative expired-arterial differences in P_{O_2} have since been found (Johansen, personal communication, 1969). Robin and Murdaugh (1967) found a small negative expired-arterial P_{O_2} difference in one out of six experiments with *Squalus acanthias*. Piiper and Schumann (1967) and Baumgarten-Schumann and Piiper (1968) reported negative expired-arterial P_{O_2} differences in *Scyliorhinus stellaris*, though not consistently, and in the present paper negative expired-arterial P_{O_2} differences in Port Jackson sharks will be reported.

Clearly there is evidence that gas exchange in elasmobranch gills cannot be accounted for solely by a simple concurrent system. Multicapillary and counter-current systems operating together or separately, appear to provide the only possible alternatives.

Piiper and Schumann (1967) suggested that the only way to differentiate experimentally between these two models would be to reverse the water or blood flow. However, all other factors, including gill geometry, must remain unaltered and this is very difficult to ensure.

In the present paper, models are derived which present the relationships between gas tensions of inspired and expired water and arterial blood for multicapillary concurrent and counter-current systems. These relationships are then compared with data from Port Jackson sharks *in vivo*.

Gas Exchange in a Serial Multicapillary System

Fig. 1 a and b show simple concurrent and counter-current systems. A multicapillary model (Fig. 1 c, d) consists of a series of respiratory units connected in parallel across afferent and efferent blood vessels. In the shark gill these respiratory units are the secondary lamellae. As respiratory water passes one respiratory unit after another oxygen diffuses into the blood. Therefore, P_{O_2} in the water gradually falls from proximal to distal end of the model, as does the P_{O_2} of blood leaving each unit. However, the mixed efferent blood can have a higher P_{O_2} than the expired water and the extent of this is explored in this section. In the individual units blood could flow in either the same direction as the flow of water (Fig. 1 c) or in the reverse direction (Fig. 1 d) so a multicapillary system can be based on units with either concurrent or counter-current flow. Both of these possibilities are very different from a simple multicapillary system which takes no account of the direction of water and blood flow. In such a system the direction of flow is, of course, quite irrelevant in the final gas tensions produced.

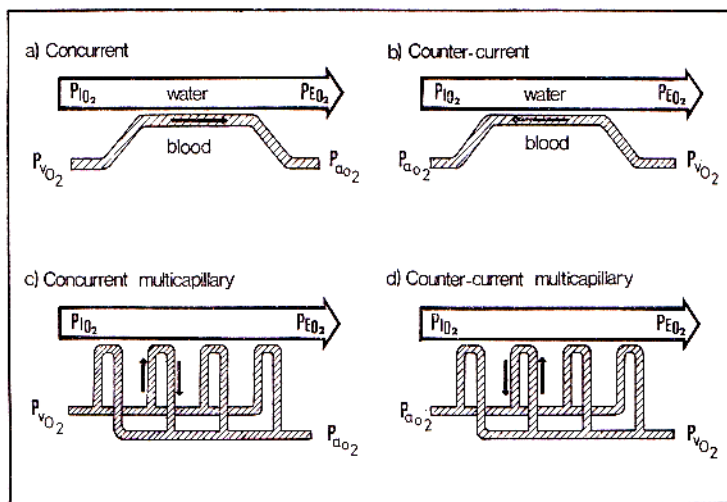


Fig. 1 a-d. Four models of gas exchange

In the models which follow, it is assumed that there is instantaneous and complete equilibration of oxygen between the blood and the respiratory medium presented to it, with no ventilation or perfusion dead spaces, no diffusion barrier, and no shunting of either blood or water past the respiratory organ. Under these conditions, $P_{aO_2} = P_{E'O_2}$ in a simple concurrent system, $P_{aO_2} = P_{I'O_2}$ in a simple counter-current system and these criteria will apply at the individual units making up multicapillary systems. In examining multicapillary systems a five unit model will be considered loading Port Jackson shark blood with oxygen from sea water. A five unit model rather than one containing, say, 200 units is adopted for arithmetical simplicity. The direction and general quantitative results are not seriously affected by taking the smaller number of units. The oxygen equilibrium curve for Port Jackson shark blood is known and it is also known that the Bohr effect in this species is negligible (Grigg, unpublished observations) so that the effect of CO_2 can be ignored.

Multicapillary System with Concurrent Units (Fig. 1c)

For each unit, or loop, with concurrent flow of water and blood (Fig. 2 a) $P_{a'O_2} = P_{E'O_2}$ (where $P_{a'O_2}$ and $P_{E'O_2}$ are the partial pressures of oxygen in the blood and water respectively as each leaves an individual unit in the model).

For oxygen uptake by each loop (P_{O_2}) we can write the following equations:

$$V'_{O_2} = V' (P_{I'O_2} - P_{E'O_2}) \cdot s, \quad (1)$$

and

$$V_{O_2} = Q' [f_1(P_{aO_2}) - f_2(P_{v'O_2})] \quad (2)$$

where

V' = volume of water ventilating that loop,

Q' = volume of blood perfusing that loop,

$P_{I_{O_2}} = P_{O_2}$ of water before that loop,
 $P_{E_{O_2}} = P_{O_2}$ of water after that loop,

$P_{a'_{O_2}} = P_{O_2}$ of blood leaving that loop,

s = solubility coefficient of oxygen in water,

f_1, f_2 = functions expressing the oxygen equilibrium curve, by which P_{O_2} can be related to percentage saturation.

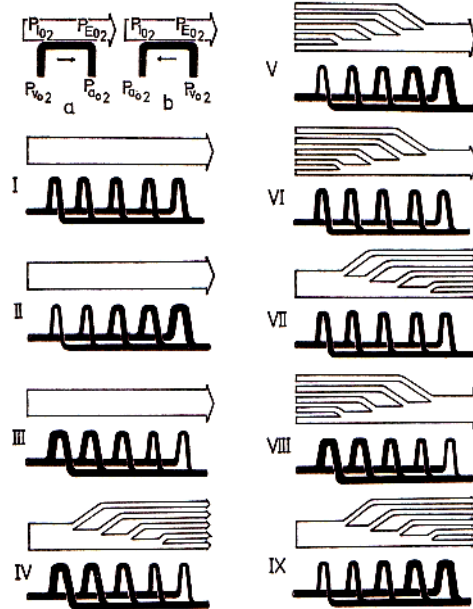


Fig. 2. Schematic representation of nine models (I-IX) of multicapillary gas exchange derived by combining different patterns of ventilation and perfusion. Such models can have concurrent units (a) or counter-current units (b)

Equating (1) and (2) we have,

$$V'(P_{I'_{O_2}}, P_{E'_{O_2}}) \cdot s = Q' [f_1(P_{a'_{O_2}}) - f_2(P_{v_{O_2}})]$$

Rearranging and substituting $P_{a'_{O_2}} = P_{E'_{O_2}}$;

If V' and Q' are known, and $P_{v_{O_2}}$ assumed, the left hand side and V'/Q' on the right hand side reduce to numerical terms for any one loop so that solving for $P_{E'_{O_2}}$ by trial and error provides a unique solution for each loop.

With this approach the overall gas exchange of any number of loops can be examined, the $P_{E_{O_2}}$ from one loop providing the $P_{I_{O_2}}$ for the next and so on. The P_{O_2} of mixed efferent blood ($P_{a_{O_2}}$) is determined by adding all the f_1 ($P_{a'_{O_2}}$) terms, dividing by the number of loops and converting that value (oxygen content) to P_{O_2} using the oxygen equilibrium curve. If total derived V_{O_2} for the whole model does not equal the standard V_{O_2} used throughout, $P_{v_{O_2}}$ is changed in appropriate directions, and the calculations repeated until appropriate values of V_{O_2} and $P_{v_{O_2}}$ are found. Thus, in each model, total V_{O_2} , $P_{I_{O_2}}$, total V and total Q are held constant. The values of $P_{E_{O_2}}$, $P_{v_{O_2}}$ and $P_{a_{O_2}}$ which emerge are then unique solutions resulting from changes in flow patterns of water and blood alone. We have explored a five-loop model in nine different water and blood flow patterns (Fig. 2, I-IX), using actual data (as yet unpublished) to provide a realistic basis for the calculations. Thus, throughout all models,

initial $P_{I_{O_2}} = 140$ mm Hg.

Total $V/Q = 15$ ($V = 4500$ mls/min; $Q = 300$ mls/min),

$V_{O_2} = 6.85 (\pm 0.07)$ mls/min

O_2 capacity of whole blood = 5.5 vols %.

$P_{v_{O_2}} = 20$ mm Hg (in the initial model)

$s = 0.000035$ mls O_2 /ml sea water/mm Hg.

The final overall picture for each model is given in Table 1. Attention is drawn particularly to values of P_{aO_2} in relation to both P_{EO_2} and $(P_{IO_2}+P_{EO_2})/2$. The models with an increasing or decreasing gradient of blood flow (II, III) achieve no increased efficiency by this means compared with the standard model (I). Furthermore, stepwise water dispersal (VII) or recruitment (VI) results in lowered efficiency as do combinations of these variations (IV, V, VIII, IX). The most efficient models appear to be those in which water flows evenly over all of the serially arranged respiratory units, with no advantage or disadvantage resulting from gradients of blood flow in either direction along the model. P_{aO_2} may considerably exceed P_{EO_2} but cannot exceed $(P_{IO_2} + P_{EO_2})/2$.

Table 1. *Results from multicapillary models with concurrent units*

Model	P_{EO_2}	P_{VO_2}	P_{aO_2}	$(P_{IO_2}+P_{EO_2})/2$
I	96.5	20.0	113.0	118
II	96.6	20.0	113.0	118
III	96.8	20.0	113.0	118
IV	96.6	19.8	106.0	118
V	96.6	19.8	106.0	118
VI	96.5	19.5	96.5	118
VII	96.6	19.2	87.6	118
VIII	96.5	19.0	77.7	118
IX	96.7	18.5	52.0	118

Multicapillary System with Counter-Current Units (Fig. 1d)

In a multicapillary system made up of counter-current units (Fig. 1 d) $P_{a'O_2}=P_{I'O_2}$ in each loop (Fig. 2b). The same equations can be written to describe oxygen uptake by the loop, that is

$$V'_{O_2} = V' (P_{I'O_2} - P_{E'O_2}) \cdot s, \quad (1)$$

and

$$V_{O_2} = Q' [f_1(P_{aO_2}) - f_2(P_{V'O_2})] \quad (2)$$

Equating, rearranging, and substituting $P_{aO_2} - P_{IO_2}$ we have,

$$P_{E'O_2} = \frac{V'/Q \cdot P_{IO_2} \cdot s - f_1(P_{IO_2}) + f_2(P_{VO_2})}{V'Q' \cdot s}$$

in which $P_{E'O_2}$ is the only unknown for each loop. Each value of $P_{E'O_2}$ is calculated, becomes the $P_{I'O_2}$ for the next loop and so the overall oxygen uptake of this type of multicapillary system can be calculated using the same values as before, derived from Port Jackson sharks. The results are given in Table 2. In comparison with multicapillary systems made up of concurrent units (Table 1), the efficiency of systems with countercurrent units is much less impaired by variations in water flow, although the same general conclusions apply. Increasing or decreasing gradients of blood flow (II, III) cause no reduction in efficiency, but stepwise dispersal (VII) or recruitment (VI) of water results in lowered efficiency, as do combinations of these variations (IV, V, VIII, IX). The most efficient models of this type also appear to be those in which an even flow of water passes over all of the respiratory units.

Table 2. Results from multicapillary models with counter-current units

Model	P _{E02}	P _{v02}	P _{a02}	(P _{I02} +P _{E02})/2
I	96.6	20.5	121.6	118
II	96.6	20.5	121.9	118
III	96.6	20.5	121.9	118
IV	96.5	20.4	120.5	118
V	96.5	20.4	120.2	118
VI	96.7	20.2	116.0	118
VII	96.7	20.2	116.0	118
VIII	96.5	20.0	114.0	118
IX	96.7	20.0	112.0	118

The theoretical efficiency of this second type of multicapillary system (Fig. 1 d) surpasses that of the first type (Fig. 1 c). P_{a02} is slightly greater than (P_{I02}+ P_{E02})/2 in some of the models (I-V). However, it is possible to calculate what degree of inefficiency would produce an end result in which P_{a02} (say 122) became less than (P_{I02}+ P_{E02})/2 say 118.

e.g. Let x =fractional effective blood shunt, combining the effects of perfusion dead space and tissue diffusion dead space. Then

$$f_2 (P_{v02}) x + [f_1(122)] (1-x)=f_1 (118)$$

i.e.

$$0.0305 x + 0.0533 (1 - x) = 0.0530; x = 0.00915 (=1 \%).$$

Alternatively, the inefficiency resulting from the effective water shunt (combining ventilation and water diffusion dead space) must not exceed 17 % before P_{a02} falls below (P_{I02}+ P_{E02})/2.

Therefore, the efficiency must be reduced by no more than 1 % of the theoretical maximum to reduce P_{a02} to equal (P_{I02}+ P_{E02})/2. For P_{a02} to exceed this average, the combination of perfusion dead space and diffusion barrier must not reduce the efficiency below 99% even if ventilation and water diffusion dead spaces are non-existent. This is an unlikely level of efficiency. Although P_{a02} can theoretically exceed (P_{I02}+P_{E02})/2 by a very small amount, the degree of efficiency required for this result is too high (99%) to be possible *in vivo*, and we propose therefore that P_{a02} can never exceed (P_{I02}+ P_{E02})/2 in a multicapillary system of this type *in vivo*.

Oxygen Uptake in Port Jackson Sharks

a) Methods

Port Jackson sharks captured in or near the entrance of Sydney Harbour were maintained at Marineland, Manly, until required. In the laboratory sharks were clamped in a stainless steel experimental tank through which filtered and aerated seawater circulated at 20°C. In some experiments sleeves made from nylon cloth were stitched and glued (Methyl 2-cyanoacrylate monomer, Ethicon) around the gill regions on both sides to allow sampling of mixed expired water. This method is similar to that described by Lenfant and Johansen (1966).

In other experiments expired water was sampled from a cannula placed in the third gill flap on the left or right side. No significant differences were found between the results obtained by the different techniques. Samples of inspired water were taken from just in front of the mouth. For sampling mixed arterial blood the upper lobe of the tail was severed to expose the dorsal aorta which was then intubated with a polyethylene cannula as far as the level of the first dorsal fin. In the caudal region the dorsal aorta tapers quite sharply so a cannula chosen to fit snugly in the severed vessel in the tail region was a loose fit a few inches anteriorly and was not obstructive. The partial pressure of oxygen in inspired water (P_{I02}), expired water (P_{E02}) and arterial blood (P_{a02}) were analysed with a Radiometer blood gas analysis system at the same temperature as the water in the

experimental tank. A more complete report on the analysis of gas exchange in Port Jackson sharks will be given elsewhere.

b) Results

The data from 12 males (4.0-7.8 kg) and 5 females (8.5-16.0 kg) consist of 104 sets of values for P_{IO_2} , P_{EO_2} and P_{aO_2} . Values in each set were measured on samples drawn sequentially over a four to five minute period from resting sharks in well oxygenated water (P_{IO_2} not less than 120 mm Hg). The frequency distribution of these values is given in Fig. 3. Each set was categorised into one of the following groups

- (i) $P_{aO_2} < P_{EO_2}$
- (ii) $P_{aO_2} > P_{EO_2}$ but $< (P_{IO_2} + P_{EO_2})/2$,
- (iii) $P_{aO_2} > (P_{IO_2} + P_{EO_2})/2$.

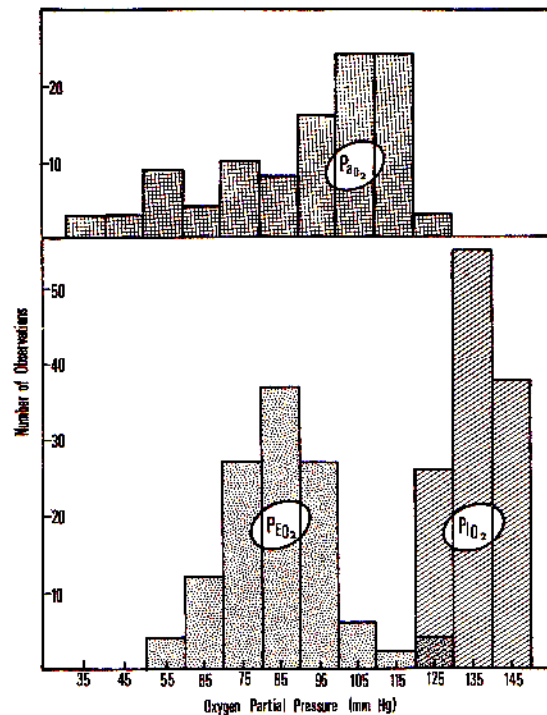


Fig. 3. Frequency distribution of 104 sets of determinations of P_{aO_2} , P_{IO_2} and P_{EO_2} to show that P_{aO_2} commonly exceeded P_{EO_2} .

The data from individual fish are shown in Table 3. The categories are not mutually exclusive because of the variability of P_{aO_2} and other parameters, presumably the result of changes in the respiratory demands of the fish. From Table 3 and Fig. 3 it is seen that P_{aO_2} frequently exceeded P_{EO_2} . Values of P_{aO_2} higher than P_{EO_2} were recorded for part or all of the time from all but three of the seventeen sharks studied (70 % of the 104 sets of observations). It is therefore clear that a negative expired-arterial P_{O_2} difference occurs commonly in resting Port Jackson sharks.

Table 3. P_{aO_2} in relation to simultaneous determinations of P_{EO_2} and $(P_{IO_2} + P_{EO_2})/2$ in Port Jackson sharks

Shark	Sex	Weight (kg)	$P_{aO_2} < P_{EO_2}$	$P_{aO_2} > P_{EO_2}$ but $< (P_{IO_2} + P_{EO_2})/2$	$P_{aO_2} > (P_{IO_2} + P_{EO_2})/2$
1	Male	5.0		Yes	Yes
2	Male	5.8	Yes		
3	Male	5.7	Yes		
5	Male	4.8		Yes	Yes
6	Male	5.6		Yes	Yes
7	Male	4.0			Yes
10	Male	4.9	Yes	Yes	
11	Male	7.8	Yes	Yes	
12	Female	12.7	Yes	Yes	
14	Female	16.0	Yes		
15	Male	5.7		Yes	Yes
16	Male	6.6	Yes	Yes	Yes
22	Male	5.7		Yes	Yes
36	Male	6.1	Yes	Yes	
38	Female	8.5		Yes	Yes
39	Female	9.3	Yes	Yes	
41	Female	12.6	Yes	Yes	

Comparison of P_{aO_2} values with the averaged oxygen tensions of inspired and expired water is analysed in Table 3 and Fig. 4. Of the fourteen sharks in which P_{aO_2} exceeded P_{EO_2} eight showed values of P_{aO_2} which exceeded $(P_{IO_2} + P_{EO_2})/2$ (47% of the 73 sets of observations in which P_{aO_2} exceeded P_{EO_2} . There appears to be no doubt from the analysis that P_{aO_2} frequently exceeds $(P_{IO_2} + P_{EO_2})/2$ in resting Port Jackson sharks. Furthermore, these values of P_{aO_2} which are not much less than $(P_{IO_2} + P_{EO_2})/2$ are also of significance because the efficiency reducing factors which tend to lower P_{aO_2} were ignored by the model but are of course present *in vivo*.

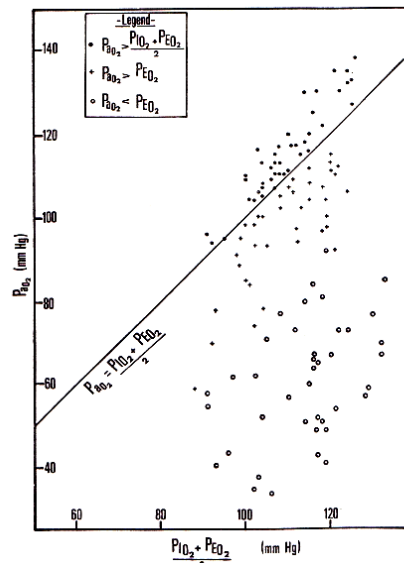


Fig. 4. Values of P_{aO_2} in relation to corresponding values of P_{EO_2} and $(P_{IO_2} + P_{EO_2})/2$. Where P_{aO_2} exceeded $(P_{IO_2} + P_{EO_2})/2$ gas exchange was too efficient to be accounted for by any serial multicapillary system

Discussion

The aim of this paper has been to examine oxygen uptake by models of multicapillary systems and to apply the predictions obtained to data from Port Jackson sharks so as to evaluate the hypothesis that elasmobranch gas exchange depends on a multicapillary system. We have established that neither a concurrent nor a counter-current multicapillary system operating at its theoretical maximum efficiency can result in P_{aO_2} higher than the average of inspired and expired oxygen tensions. Yet P_{aO_2} frequently exceeds $(P_{IO_2} + P_{EO_2})/2$ in Port Jackson sharks.

There is therefore no doubt that a functional counter-current system operates in these sharks. It now remains to examine how this occurs and to discuss the situation in the shark gill.

We agree with Piiper and Schumann (1967) that the anatomy of the gills suggests a multicapillary system, containing numerous blood filled respiratory units arranged in parallel across the afferent and efferent blood vessels. Particular attention should be paid to Fig. 5. In a multicapillary system water flows sequentially past the series of respiratory units which are therefore exposed to a falling P_{IO_2} along the length of the organ. This is the way in which Piiper and Schumann envisaged water flow in sharks; "... the respiratory water may have to flow along the length of the gill filaments attached to the septal wall..." (Fig. 5a). However, Grigg (1970) has shown in *Heterodontus* that water flows between the secondary lamellae and into the septa] canals between the bases of adjacent filaments and thence to the exterior (Fig. 5b). In this way, each secondary lamella functions as an independent counter-current unit exposed to water of high oxygen tension (P_{IO_2} , or close to it), and not to water that has already given up oxygen to the more proximal units on the filament. Herein lies the essential point.

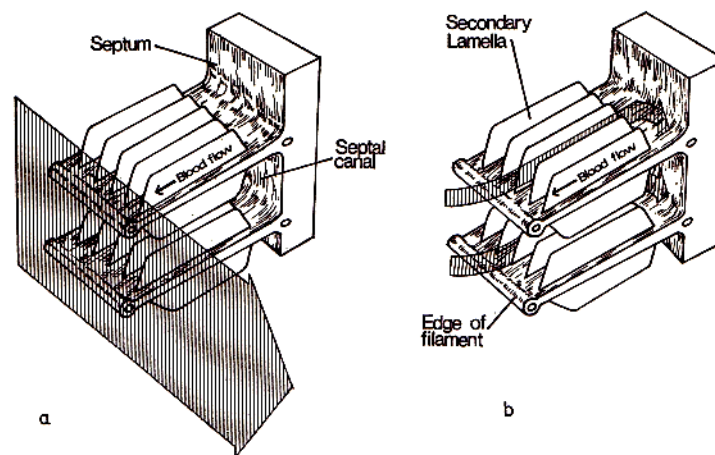


Fig. 5a and b. The two models compared. Piiper and Schumann (1967) proposed that the secondary lamellae are the units of a multicapillary system and that P_{O_2} of the water falls as it passes along the length of each filament (a). In the present model (b) water flows between the secondary lamellae each of which functions as an independent counter-current unit exposed to water of high oxygen tension

It is not suggested that the gill surfaces do not contain multiple capillaries, but that their arrangement is such that from a functional viewpoint they provide a counter-current and not a multicapillary system.

The operation of a functional counter-current in the gills of Port Jackson sharks is now established. Water does flow between the respiratory units of the gills in an opposite direction to blood flow and the physiological considerations outlined in the present paper show that this counter-current is indeed a functional one. Without their raw data it is difficult to tell if P_{aO_2} frequently exceeded $(P_{IO_2} + P_{EO_2})/2$ in Piiper and Schumann's study of *Scyliorhinus stellaris*. They did report some very high values of P_{aO_2} and it may well be that the application of this criterion to their data would establish the operation of a functional counter-current in that shark also. Certainly the gill structure of all elasmobranches we have looked at is similar to that of *Heterodontus* and the presence of the septal canals seems to be a general feature. There is no reason to suppose that the conclusions about gas exchange in *Heterodontus* are in any way atypical and the gills of all elasmobranches may operate similarly.

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