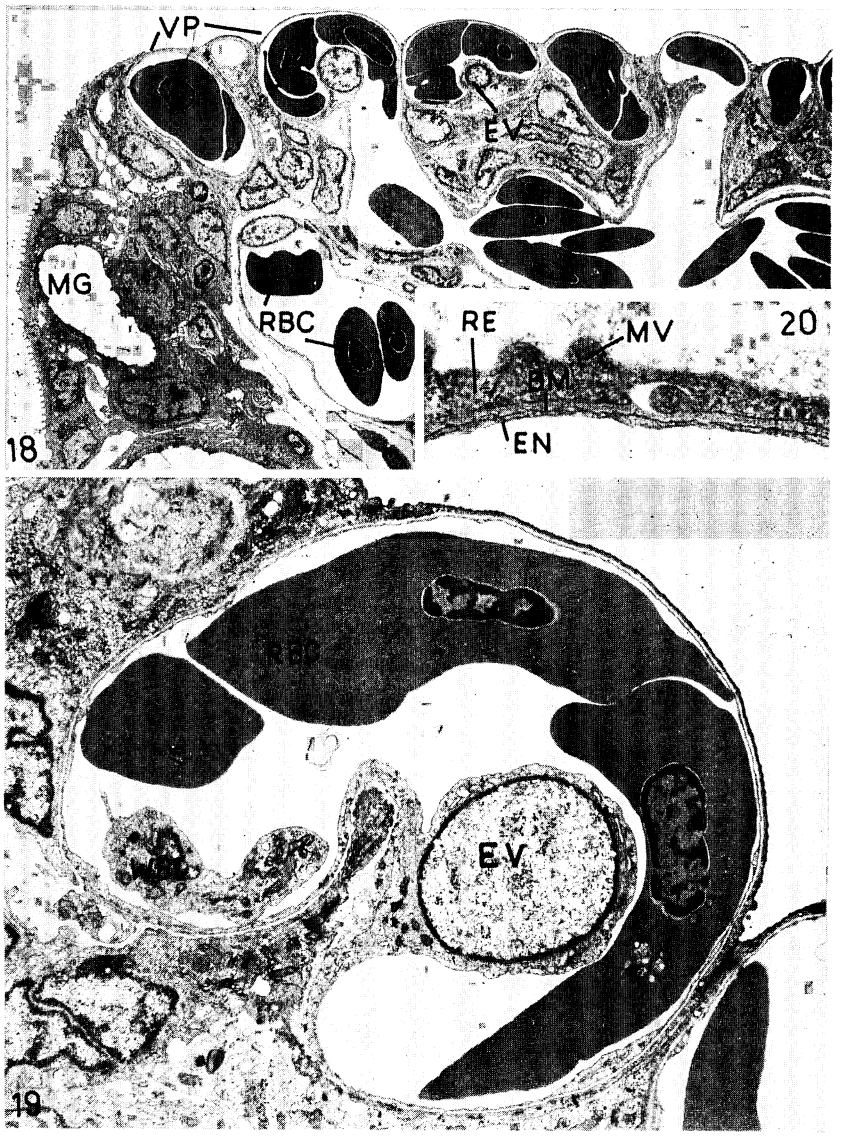

Fig. 18. Vertical section passing through the air sac showing vascular and non-vascular areas and connective tissue region with the arterial and venous supply to the respiratory vascular papillae. ×1,800. EV, endothelial valve; MG, mucous gland; RBC, red blood corpuscle; VP, vascular papilla.

Fig. 19. TEM of a vertical section of a respiratory vascular papilla showing endothelial valve (EV), red blood corpuscle (RBC), white blood corpuscle (WBC) and the thin tissue barrier. ×8,000.

Fig. 20. High magnification of the tissue barrier of a respiratory vascular papilla showing three distinct layers—the respiratory epithelium (RE), basement membrane (BM) and endothelium (EN), microvilli (MV). ×56,000.

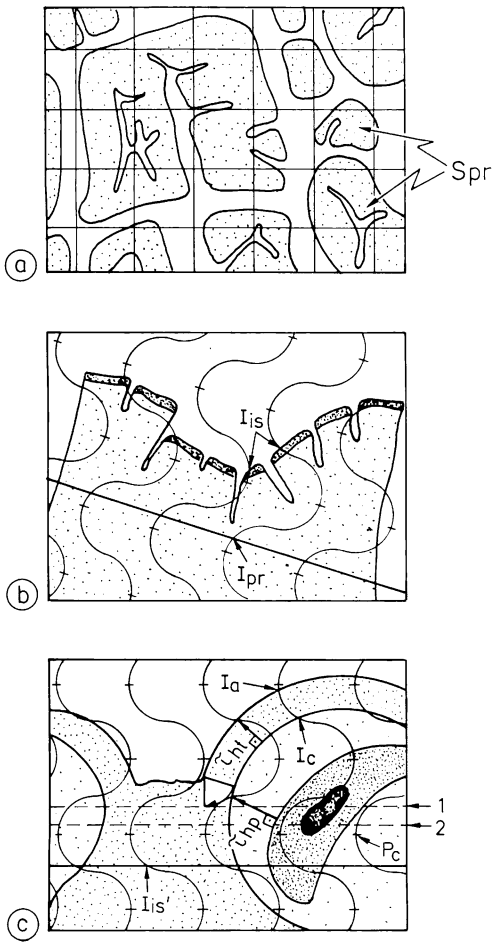

Fig. 21. Diagram to illustrate morphometric models and symbols. a: Part of respiratory area (projected area of respiratory membrane) subdivided into islets and non-respiratory regions (I_{is}=intersection of grid with islets; I_{pr}= intersection with projected plane). b: Light microscopic section showing intersections of grid with islets (I_{is}) separated by non-vascularised areas and intersections (I_{pr}) with projected plane. c: EM level with intersections of outer surface (I_a) and capillary (I_c), and points falling on capillarys (P_c). I_{is}', intersection with islets (EM level); τ_{ht}, measurement for thickness of tissue barrier; τ_{hp}, measurements for thickness of plasma layer.

ing the pharynx, hypopharynx and air sacs was dissected from fishes of about 200 g and photographed. By planimetry the entire surface area of the membrane was measured. About 1/4 of the total respiratory membrane is occupied by respiratory islets.

The basis of the model and measurements used in this study is shown in Fig. 21 which was designed to obtain information about: a) the proportion of the respiratory and non-respiratory area in the organ; b) the proportion of respiratory and non-respiratory tissue in the islets; c) the relative size of the surface of the papillae and of the epithelial and blood tissues. As can be seen from Fig. 21b, the respiratory surface is not flat and the present model takes into account this folding and breaking up into islets and the curvature of the outer surface of the papillae (Fig. 21c).

The analysis was carried out in three stages: (i) the first used the dissected air sac and other respiratory surfaces and by point counting the proportion of the total surface occupied by respiratory islets and their absolute area made it possible to calculate the projected area (S_{pr}).

ii) Secondly from light microscopic sections on which a Mertz grid had been superimposed, intersection counts were made of the folded islet surface (I_{is}) relative to intersections with a straight line parallel to the surface (I_{pr}), which was taken to represent the projected area. In this way the ratio I_{is}/I_{pr} was calculated.

iii) The third level of the analysis used electron micrographs which were projected onto a screen containing a Mertz grid so that intersections with the outer epithelium (I_a) were estimated relative to an equivalent islet area (I_{is}') and hence the factor I_a/I_{is}' was estimated. The EM sections were also used for counting the intersections of the capillary (Ic) and the points falling on the capillaries (Pc) of the papillae.

From these measurements the overall calculation of the surface area of the outer epithelial surface is given by the relationship: $S_a=S_{pr}\cdot I_{is}/I_{pr}\cdot I_a/I_{is}'$. These calculations gave values of 20.0 cm^2 for the total respiratory area of the 200 g fish. The area of the capillary surface (S_c) is almost identical to S_a because the barrier is extremely thin.

The volume of capillaries $(V_c)=S_{pr}\cdot I_{is}/I_{pr}\cdot P_c/I_{is}'\cdot d/4$, where d is the repeating dimension of the grid (Fig. 21). Insertion of determined values gave: 5.44×10^{-3} cm^3. Hence the capillary loading of the surface, estimated by $V_c/S_a=5.44\cdot10^{-3}$ $cm^3/20.0$ cm^2=amounts to 2.72 cm^3/m^2.

Barrier thickness. Using the EM projections with the Mertz grid, distances were measured from

intersections of the grid lines with the particular surface, i.e., perpendicular to the endothelial/blood surface or to a red cell in the case of measurements of plasma thickness. Measurements were made of total tissue barrier (epithelium, basement membrane, endothelium), plasma layer and outer mucus layer separately. The values of harmonic mean thickness (τ_h) of these layers were as follows: Tissue 0.72×10^{-4} cm; plasma 0.19×10^{-4} cm; mucus 1.5×10^{-4} cm (rough estimate).

Diffusing capacity. Calculations of the individual diffusing capacities are set in Table 1 where values are given using two different Krogh permeation coefficients for oxygen (K_1) for mammalian tissue and (K_2) for other air-breathing fishes. Thus two values for DL (ml O_2/min/mmHg/kg) were obtained 0.00165 and 0.0008 respectively.

Discussion

Gills. In *Monopterus cuchia*, the gills are very much atrophied, the second branchial arch only bearing a few small finger-like filaments. Near the base, the filaments and lamellae fuse with each other to form pores or slits through which water passes during aquatic ventilation. They represent interlamellar spaces. The significance of these slits is not very clear but similar structures are present in other air-breathing fishes, e.g., *Anabas* sp. and *Channa striata* (Munshi, 1968, 1976). The secondary lamellae are represented by small bud-like structures having vascular coils that are lined internally by endothelial cells (Hughes and Munshi, 1979).

Air-breathing organs. The air sacs are posterodorsal extended pouches of the pharynx. New respiratory islets develop as small protruding buds in between old islets in the non-vascular lanes of of the air sac and buccopharynx. The posterior one third of the sac is non-vascular and perhaps serves as a reservoir for residual air. It is not known whether the fish in normal conditions uses its buccopharynx for aerial respiration, as between air-breaths it ventilates water through the buccopharynx and gills. Regular gill filaments and lamellae are lacking but it is possible that respiratory islets on the inner surfaces of the buccopharynx, hypopharynx (near gullet) and over the gill arches function in aquatic gas exchange and are also used in aerial respiration, when the fish ventilates air.

The versatility of this surface of *M. cuchia* is remarkable as it functions not only for gas exchange in both air and water but also for the passage of food (earthworms, molluscs, insects, crustacea) of the fish. The respiratory hypopharynx is a small chamber situated just behind the pharynx leading into the oesophagus. The internal lining is highly vascularised and beset with respiratory islets. The entire respiratory membrane is covered over by a protective mucous layer which is anchored by microridges on the outer epithelial surface. Large numbers of unicellular mucous glands present in the non-vascular areas of the respiratory membrane actively secrete mucus. The mucus makes the surface slippery so that the fish can suck in its prey, which passes over the surface of the respiratory epithelium without causing any damage to the system. Sense organs (chemoreceptors) were also found in the non-vascular areas of the air sac.

The experiments conducted by Lomholt and Johansen (1976) established that this gas exchange system could extract oxygen from both water and air. The aquatic oxygen uptake increased by 150% when air-breathing was prevented. They attributed this to the latent ability of the gills and buccal mucosa to absorb oxygen from water; or perhaps it reflects a relative increase in cutaneous oxygen uptake (Mittal and Munshi, 1971). However, the compensatory increase did not suffice to maintain the overall oxygen demand. This is mainly because the gills are atrophied and the low diffusing capacity of the respiratory islets.

Lomholt and Johansen (1976) observed that sometimes the fish stayed voluntarily submerged for an unusually long period and pumped water in and out of the air sacs. The rate and mode of these movements resembled those of air-breathing and were unlike the shallow, unidirectional pumping of water across the gills of fish denied access to the surface (Lomholt and Johansen, 1974). In this species Hora (1935) also observed this type of ventilation of the air sacs with water. This mechanism is possible because there are no shutters to close the inhalent apertures. Alternate replacement of air and water during air ventilation have been observed in other air-breathing fishes, e.g., *Osphronemus* sp. (Peters, 1978; Hughes, 1978a), *Channa argus* (Ishimatsu and Itazawa, 1981).

The ventral aortic blood pressure fell from about 60 mmHg systolic value to 40 mmHg in the

Table 1. Subdivisions of the diffusing capacity (ml O_2/min/mmHg) of air-breathing organs in *Monopterus cuchia* (200 g), based on morphometry. Results are given using two different values for the Krogh permeation coefficient ($K_1=3.3\times10^{-6}$, $K_2=1.5\times10^{-6}$ ml O_2/min/mmHg/mm²/μm).

	K_1	K_2
D_t ($K\cdot S_a/\tau_{ht}$)	9.17×10^{-3}	4.17×10^{-3}
D_p ($K\cdot S_c/\tau_{hp}$)	3.47×10^{-2}	1.58×10^{-2}
D_e ($\theta\cdot V_c$)	2.75×10^{-3}	
D_{mu} ($K(S_a\times0.9)/\tau_{hmu}$)	3.96×10^{-4}	1.8×10^{-4}
D_L	3.304×10^{-4}	1.607×10^{-4}
D_L without mucus	1.99×10^{-3}	1.5×10^{-3}
D_L without mucus and RBC	7.25×10^{-3}	3.3×10^{-3}
D_t tissue only	9.17×10^{-3}	4.17×10^{-3}

dorsal aorta indicating considerable vascular resistance in the shunt connecting these vessels (Lomholt and Johansen, 1976; Satchell, 1976). The endothelial cell complex present in each vascular papilla of the respiratory islets must affect movement of erythrocytes through them. Perhaps they work as minute valves which can control the resistance to blood flow. The space between the endothelial cell complex and the respiratory surface of the vascular papilla is so minute that at a time only one RBC could pass through. The size of the erythrocytes varied from 12.8 μm×9.7 μm to 15.5 μm×9.9 μm and the calculated average number of erythrocytes per mm³ of blood is 2.03×10^6. The haematocrit value is high (range 40–54%, Mishra et al., 1977).

Diffusing capacity. The model adopted in the present study is more appropriate to the detailed anatomy of air-breathing organs and has enabled correction to be made to previously published values obtained for a larger number of specimens. A summary of some of the results obtained for

Table 2. Comparison of values for D_t and D_L obtained in the present study with those of other air-breathing fishes and the laboratory white rat. Results are given in two columns according to the value of K used in the calculations ($K_1=3.3\times10^{-6}$, $K_2=1.5\times10^{-6}$ ml O_2/min/mmHg/mm²/μm)

	K_1	K_2	
D_t (ml O_2/min/mmHg/kg)			
Monopterus cuchia	0.0458	0.02085	Present study
Monopterus cuchia (air sac only)		0.0165	Hughes et al. (1974b)
Heteropneustes fossilis (air sac)		0.0288	Hughes et al. (1974a)
Channa punctata		0.0753	Hakim et al. (1978)
Anabas testudineus (Suprabranchial chamber+labyrinthine organ)		0.28	Hughes et al. (1973)
D_L (ml O_2/min/mmHg/kg)			
Monopterus cuchia	0.00165	0.0008	Present study
Lepidosiren paradoxa	0.235		Hughes and Weibel (1976)
Protopterus aethiopicus	0.158		Maina and Malloiy (1985)
White rat (140 g)	3.83		Gehr et al. (1981)

Table 3. Comparison of surface area (S_a), diffusing capacity (D_L, D_t) and resting oxygen consumption ($\dot{V}o_2$) of *Monopterus cuchia* with corresponding data for small mammals.

	S_a (cm²)	D_t (ml O_2/min/mmHg/kg)	D_L	$\dot{V}o_2$ (ml O_2/sec/kg)	$\dot{V}o_2/D_L$ (mmHg)
M. cuchia (200 g) Present study	20.0	0.02085	0.00165	3.5×10^{-3}	127.3
White rat (140 g) Gehr et al. (1981)	4070	—	3.83	1.61	25.2
Mouse (22.8 g) Geelhaar and Weibel (1971)	677	21.0	6.4	0.75	7.03

surface area, thickness and diffusing capacity of the respiratory surface are given in Table 1 and those for some other fish in previous studies in Table 2. From this table it is evident that the diffusing capacity of the tissue barrier (Dt) obtained in the present results is about 25% greater than that obtained for the same species in 1974 (Hughes et al., 1974b) but overall diffusing capacity (D_L) is 10 times smaller because of taking into account the mucus (D_{mu}), plasma (D_p) and erythrocyte (D_e) components in this study. In the previous study no account was taken of respiratory membrane of the pharynx and hypopharynx but an over estimate was made of the air sac area because the whole of the inner surface was assumed to contain respiratory islets. Though the surface area of the total respiratory membrane was found to be almost double (20 cm^2) the value obtained previously for air-sacs alone (9.50 cm^2) in a fish of 200 g, the tissue barrier together with the mucous and plasma layers was found to be 2.41 μm, which is almost six times greater than values used in the earlier study (0.44 μm). This resulted in the overall ten fold reduction in its diffusing capacity (0.00165 ml O_2/min/mmHg/kg). The value for D_L calculated in the present study (0.00165 ml O_2/min/mmHg/kg) is small relative to the other air-breathing fish in which such measurements have been made (*Lepidosiren* and *Protopterus*) and is much smaller than that of mammals of comparable size. D_t has also been estimated for other air-breathing fishes; values obtained for *Heteropneustes* were slightly greater whereas that of *Channa* is nearly four times greater and that of *Anabas* approximately 14 times greater. As is to be expected from this morphometric data the oxygen consumption of *M. cuchia* is far less than a small mammal (Table 3), but even so it would appear that the pressure difference ($\dot{V}o_2/D_L$) required to maintain this oxygen supply is high. It is a very sluggish fish.

Capillary loading (V_c/S_a). Capillary loading, which gives information about the volume of blood relative to the surface available for gas exchange, is larger in fish than mammals. In *Monopterus* it is about 2.5 times greater than an average for mammals of the same body weight (regression equation of Gehr et al., 1981) but is 1/5th that of *Lepidosiren* which has larger red cells and capillary volume (Hughes and Weibel, 1976). The large capillary loading of fish lungs and air sacs is partly due to gas exchange being restricted to one side of the capillary, but in some cases (Hughes, 1978b) this is compensated by added curvature of the air/blood barrier. The *Monopterus* respiratory papillae are a good example of this type of adaptation.

Structural evolution of vascular papillae. The vascular papillae are specialized blood capillaries connecting the branchial arterial system with those of veins of the jugular system for gas exchange. In *Monopterus*, *Channa* and *Anabas* oxygenated blood from the capillaries of the air-breathing organs returns to the anterior cardinal vein or its branches.

In *A. testudineus* the respiratory islets are composed of a series of parallel blood capillaries, eachc apillary being made up of a single row of endothelial cells. Their prominent cell bodies project into the capillary lumen. The endothelial cells have tongue-like processes which may act as minute valves controlling the flow of blood (Hughes and Munshi, 1973b; Munshi et al., 1986). These parallel capillaries are separated from each other, not by pillar cells but by epithelial cells. In *M. cuchia* these capillaries have become more complex due to their spiral disposition giving rise to the vascular papillae in series. At each turn of the spiral the epithelial cells along with the basement membrane get tucked into the bases of the curvature of the capillary forming the vascular papillae. At these points the endothelial cells develop enormously to take up the role of valve like structures much like those of *Anabas* and *Channa*. These endothelial valves are metabolically active with mitochondria and vesicles. The structural similarities of the vascular papillae and their similar disposition in the buccopharynx both in *Channa* sp. and *M. cuchia* are very much apparent (Hughes and Munshi, 1986). At the same time while *Anabas* and *Channa* have similar gill structure with their characteristic shunt vessels, *M. cuchia* has lost functional gills. The structural similarities found in the micro-circulatory system of air-breathing organs in the three species of fishes studied suggests a morphological series, but whether it represents an evolutionary sequence is conjectural but at least represents one possibility.

The *Anabas* type may represent one of the first steps in the evolution of air-breathing organs in the Percomorphi group of fishes, where the arterio-venous connections are straight endothelial tubes situated just below the epithelium. In the second

stage they became arranged in a wave-like fashion as in *Channa* sp. In the 3rd stage, as in *M. cuchia*, the capillaries take the form of spirals. This spiral type of capillary also exists in the gill system of *M. cuchia*. The functional significance of these wave- or spiral-like arrangements of capillaries seems to be that more blood could be accommodated in a small space of respiratory islets. Moreover, the system develops an efficient mechanism to bring every individual RBC into close contact with the respiratory surface for gas exchange. It also provides enough resistance to increase their residence time and ensure proper oxygenation.

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(JSDM: Department of Zoology, Bhagalpur University, India; GMH: Unit for Comparative Animal Respiration, Bristol University, England; PG and ERW: Department of Anatomy, University of Bern, Switzerland).

合鰓類タウナギの空気呼吸器官の構造

Jyoti S. D. Munshi • George M. Hughes • Peter Gehr • Ewald R. Weibel

タウナギの空気呼吸器官の構造を，光顕，走査ならびに透過電顕によって観察し，本種における口腔咽頭呼吸に関する形態的基礎を明らかにした．気嚢（上咽頭室）は吸・出水口の役を果す開口部をもち，その 2/3 に形と大きさの異なる呼吸島をそなえている．呼吸島は水呼吸に役割を果すが，水呼吸だけではタウナギは生活できない．血管分布のないところは，微小堤をそなえた上皮細胞で覆われている．呼吸器官へは細動脈が深く侵入し，呼吸島に特有の血管乳頭を形成する．血管乳頭も，特殊化した上皮で覆われている．第2鰓弓は，多角状微小堤をもつ上皮で覆われた少数の指状弁をそなえている．立体法並びにタウナギ相応の新しい範例をとり，空気呼吸器官の形態計測を行なった．そして，体重 200 g の呼吸膜における呼吸面積，毛細管負荷などを算出した。