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## INTRODUCTION

Fish are the largest class of vertebrates; with over 21 000 existing species they outnumber the amphibians, reptiles, birds and mammals put together. Fish span a long period of geological time for the earliest hagfish are now believed to have arisen in the Cambrian period, (Hardisty 1982). As inhabitants of both salt and fresh water they are spread over 71% of the earth's surface. The greatest depths of the oceans are deeper than the world's highest mountains, so fish span a greater vertical range than air-breathing vertebrates. For this reason they endure a larger range of pressures; on land the pressure at 9000 m is 0.3 atm but in the greatest depths of the oceans it is 1200 atm. They can live continuously in waters of extreme temperature. Some of the Chaenichthyidae live permanently below the polar ice at temperatures of  $-1$  to  $-1.8$  °C; a cichlid *Oreochromis grahami* lives in alkaline lakes in Kenya's rift valley, in which the water has a temperature of 43 °C and a pH of 9.6–10.5 (Johansen *et al.* 1975). Fish have become adapted to environments quite as extreme as any occupied by higher vertebrates.

Fish, like other vertebrates, possess a circulatory system consisting of a pump, the heart, and a continuous system of branching tubes, the arteries, arterioles, capillaries and veins, which form a closed circuit so that all of the blood leaving the heart is returned to it. The blood flowing through it carries oxygen from the gills to the tissues, carbon dioxide to the skin and gills to be eliminated, the soluble products of digestion from the gut to the liver, more processed nutrients from the liver to the tissues and ions such as  $\text{Na}^+$  and  $\text{Cl}^-$  which are crucially involved in osmoregulation. It carries hormones and vitamins; certain specialized carrier proteins occur in the plasma, such as transferrin, which transports iron, and caeruloplasmin, which transports copper.

The primary role of the circulatory system is thus to maintain a flow of blood through the gills and tissues of the body, and to return it to the heart. The flow of blood through a system of tubes is governed by the same laws as operate in physical systems. The structure and physiology of any circulatory system reflects the primacy of these laws.

*Some elementary haemodynamics*

Flow, denoted by the symbol  $F$ , is measured in units such as  $\text{ml min}^{-1}$  and is determined by pressure  $P$ , and resistance  $R$ , in a manner analogous to Ohm's law.

$$F = \frac{\Delta P}{R}$$

In the equation above, pressure is shown as  $\Delta P$ , to indicate that it is the difference of pressure along the length of the vessel that effects flow. In a short length of tubing this would be equal to  $P_1 - P_2$ , i.e. the pressure difference between the two ends of the tube. The absolute pressure to which the system is subjected is irrelevant. Fish in the ocean depths are at very great pressures but these affect tissues and blood vessels alike and do not result in a gradient of pressure along particular vessels.

A further distinction needs to be made between driving pressure and transmural pressure. Pascal's first law tells us that any one level pressure acts equally in all directions. Inside a blood vessel there will be a component acting along its length; this is the  $\Delta P$  indicated above and provides the driving pressure that effects flow. A component at right angles to this exerts a pressure on the vessel wall and is opposed, to a greater or lesser extent, by a pressure exerted by the wall and the tissues that press against it. The difference between the two is the transmural pressure; if it is positive it will distend the vessel to an extent dictated by the elasticity of its wall. If it is negative, the vessel will collapse, unless, as happens in the caudal vein and some of the cutaneous veins, this is prevented by the rigidity of the wall and surrounding tissues.

Driving pressure and transmural pressure are really independent. Any restriction to the outflow from a vascular bed will raise the pressure in its veins, capillaries and arteries alike. The transmural pressure will rise accordingly but there may be little or no change in driving pressure. Increase or decrease in transmural pressure has the potential to dilate or contract vessels. Teleosts have the ability to constrict their efferent gill vessels in response to hypoxia; this raises the transmural pressure in the gill lamellae and, by dilating them, increases the area available for gas

exchange (Nilsson and Pettersson 1981). Elasmobranch fish have a prominent sphincter surrounding the opening of each hepatic vein into the sinus venosus. Johansen and Hanson (1967) report that this is dilated by catecholamines, which (Chapter 10 and 11) are liberated into the blood of elasmobranchs during exercise and hypoxia. Dilating the hepatic vein sphincters will allow the transmural pressure in the large sinuses of the hepatic and hepatic portal veins to fall and blood to be transferred from the visceral to the somatic circulation.

#### *The Poiseuille equation*

Flow through a tube may be pictured as the movement in it of concentric laminae of fluid, with the central core moving most rapidly, and the outermost layer stationary against the wall. All the resistance to flow is caused by the resistance to sliding of the laminae of fluid, one upon another. It is not, at the velocities of flow likely to be present in the vessels of fish, due to friction against the wall of the vessel. Because narrow tubes have a smaller proportion of their volume in the faster-moving axial stream, resistance is greatly influenced by the radius of the tube,  $r$ , as well as by its length,  $L$  and by the viscosity of the fluid,  $\eta$ . Poiseuille's equation tells us that

$$F = \frac{\Delta P \pi r^4}{8L\eta}$$

Because flow is proportional to the fourth power of the radius, small changes in this cause large changes in flow. A reduction in the radius of a vessel by 16% will halve the flow through it. Poiseuille's equation strictly applies to flow through rigid unbranched tubes, and is thus not entirely appropriate to the fish blood system. The arteries that take blood to the gills, for example, are not rigid, but very compliant as they are rich in elastic tissue; moreover, they branch repeatedly.

#### *Viscosity*

Blood is a fluid and a fluid may be defined as a substance which cannot indefinitely withstand a force tending to deform it and to cause one layer of it to slide over another; i.e. a shearing force. This tendency to change shape is termed viscosity,  $\eta$ , and Sir Isaac Newton in the *Principia* of 1687 defined it, with perfect clarity, as a lack of slipperiness ('*defectus lubricitatis*') between adjacent layers of fluid. Resistance to shear depends on the rate of change of deformation, the shear rate, not on the actual distance moved. The shear rate is the gradient of velocity and is expressed in units

per sec; it is derived from  $\text{cm sec}^{-1} \text{cm}^{-1}$ . The unit of  $\eta$  in the CGS system is the poise and is defined as the tangential force per unit area ( $\text{dynes cm}^{-2}$ ) when the shear rate is unity i.e.  $1 \text{ cm sec}^{-1} \text{cm}^{-1}$  and, in the haematological literature, the viscosities of bloods are normally expressed as so many centipoises, (0.01 poise). I have therefore retained this unit, from the CGS system, rather than the SI unit of Pascal seconds (Pa sec); one centipoise =  $1 \times 10^{-3}$  Pa sec. Water at  $20^\circ\text{C}$  has a  $\eta$  of approximately 1 centipoise, (1 cp): the bloods of fish have  $\eta$  values within the range of 10–90 cp. Fluids such as saline, in which  $\eta$  is independent of the shear rate, are termed Newtonian fluids. Blood has cells in it, and the large protein molecules in the plasma tend to attach to these and link them together when the blood is flowing slowly. Hence the  $\eta$  increases as the rate of shear decreases: it is an example of a non-Newtonian fluid. The  $\eta$  of blood also increases as its content of red cells is increased. As all of the resistance to flow of the blood is due to its viscosity, we can see that there may well be an optimal haematocrit, i.e. red cell content. Increasing the number of red cells will increase the capacity of the blood to transport oxygen, but will also increase the work the heart has to do to propel it through the circulatory system; indeed when the haematocrit exceeds 70%, blood can hardly be regarded as a fluid. We will return to this topic in Chapter 4.

*Total fluid energy*

Poiseuille's equation is an over-simplification in that it omits the fact that a column of moving blood has kinetic energy; at any particular point in a blood system, pressure energy is not the sole determinant of flow; the energy of the moving mass of blood needs also be considered. We can include this by stating that flow depends on differences, along the length of a vessel, in the total fluid energy of a unit volume of blood (Hicks and Badeer 1989). The pressure component in this is commonly the largest, but in low pressure, e.g. venous, systems the kinetic component may be a considerable proportion of the total. The equation is

$$E = P + pgh + \frac{1}{2}pV^2$$

$E$  = total energy in  $\text{Joules cm}^{-3}$ ,  $P$  = pressure in mm Hg,  $V$  = velocity in  $\text{cm sec}^{-1}$ ,  $p$  = density in  $\text{g cm}^{-3}$ ,  $g$  = acceleration due to gravity =  $980 \text{ cm sec}^{-2}$ ,  $h$  = height in cm above a datum level

The term  $pgh$  may be quite large in a land vertebrate, in which the height of the body above the ground may be considerable, but  $pgh$  is likely to be very small in a fish. This is because most fish have their greatest dimen-

sion, i.e. length, horizontal and because the gravitational pressure of the blood is largely cancelled out by the buoyancy supplied by the surrounding water. But it may be that during sudden accelerations and decelerations transients are developed in which the acceleration due to gravity is replaced briefly by forces generated by the movement of the fish. In the blue fish, *Pomatomus saltatrix*, the acceleration is up to 3G and Ogilvy and DuBois (1982) suggest that such forces could have led, in the course of evolution, to a toleration of the force of gravity.

Flow will occur along a blood vessel from A to B if the total energy, at the point A exceeds that at B, and in certain situations, such as the base of the mammalian aorta, flow may be against the gradient of pressure. The two terms,  $P$  and  $\frac{1}{2}\rho V^2$  are to some extent interconvertible. The complex fourth chamber of the teleost heart, the bulbus arteriosus, (Chapter 2) is, it seems, a device for converting kinetic energy to pressure energy during systole and changing it back again during diastole. As we shall note shortly, the same volume of blood has to flow through each segment of the circulatory system but the proportions of the pressure and kinetic terms are widely different in the arteries and veins. In the central veins, where pressures are ambient or even subambient, kinetic energy must be a substantial part of the total, although we as yet lack the data to say what this fraction is.

#### Resistance

Resistance may be calculated by dividing pressure by flow and thus has arbitrary units of mm Hg ml<sup>-1</sup> (of flow) min<sup>-1</sup> kg<sup>-1</sup> (of fish body weight). The greater the pressure required to propel 1 ml of blood through the vascular bed, the higher the resistance must be. Work done against resistance is ultimately dissipated as heat and is lost to the system. There are thus advantages in keeping the total peripheral resistance, TPR, as low as possible. When resistances are connected in series, the TPR is simply the sum of their separate values.

$$\text{TPR} = R_1 + R_2 + R_3 + \dots R_n.$$

When resistances are connected in parallel the reciprocal of the TPR is the sum of the reciprocals of their separate values.

$$\frac{1}{\text{TPR}} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} + \dots \frac{1}{R_n}$$

Clearly, the 'in parallel' arrangement results in a much lower TPR. Three vascular resistances each of 100 resistance units have, in series, a TPR of

300: in parallel they have a TPR of 33.3 units. Fish show the basic metameric structure of vertebrates more evidently than do the tetrapods; the trunk consists of a series of rather similar segments. Blood flows into each of these from segmental arteries branching from the dorsal aorta, and is returned in segmental veins. Each of these segmental circulations is in parallel, and all together are in parallel with the circulation of the gut and other viscera. The separate circulations of each of the gills offer another example. Not only are the eight gills of a 51 g whiting, *Gadus merlangus*, in parallel with each other but so are the 72 000 respiratory lamellae (Hughes 1966), which such a fish is likely to possess. A blood cell may pass through any one, but only one, of these in its journey round the body.

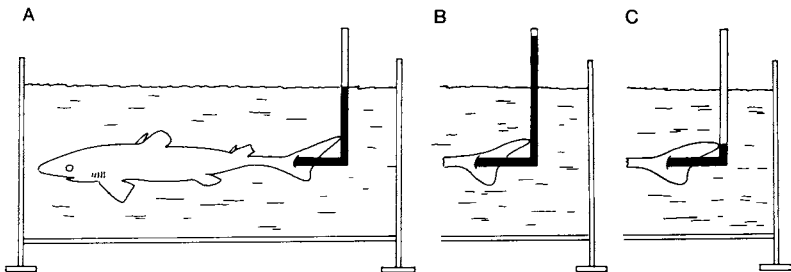
In addition to reducing the TPR, the arrangement has two other advantages. Firstly, each vascular bed is assured of a supply of fresh blood from which nothing has been withdrawn and to which nothing has been added. Secondly, the 'in parallel' arrangement facilitates circulatory regulation. Restricting flow to one or more vascular beds makes blood available for other regions. As we shall see in Chapters 10 and 11, maintaining a relatively constant arterial pressure and decreasing the resistance to flow into active tissues has been the strategy for circulatory control adopted by all fish, and indeed by other vertebrate groups.

There are circumstances when 'in series' systems are of value; they occur when one vascular bed requires blood to which some agent has been added or removed by a previous one. The gills are in series with all the other tissues of the body and fish differ from all the land vertebrates in this respect. Thus the heart has only a single ventricle, and the blood is not returned to have its pressure raised a second time, as it is in reptiles, birds and mammals. Oxygen is added to the blood prior to its passage to the tissues, but between a half and a third of the blood pressure generated by the heart is lost in overcoming the resistance of the branchial circuit. The hepatic portal circulation carries the soluble products of digestion such as amino acids and glucose forward to the liver to be reformulated into proteins and glycogen. The renal portal circulation is intercalated between the vascular beds of the post abdominal trunk, and the capillary vessels of the kidney tubules. Through these, divalent ions such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are excreted and necessities such as proteins and glucose are retrieved. In these situations we must surmise that the advantages of these localized transport systems outweigh the loss of pressure which resistances in series impose.

*Pressures on land and in water*

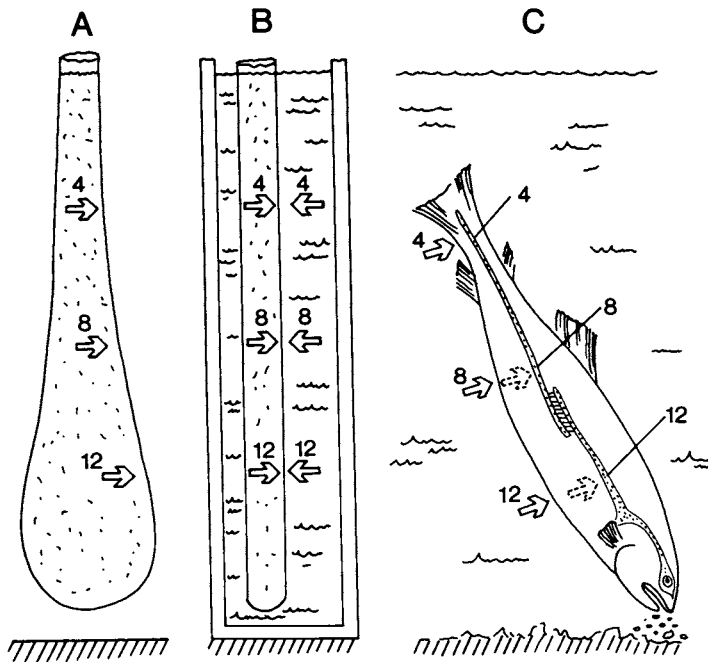
Measurements of blood pressure in terrestrial vertebrates are normally expressed as so many units of pressure, mm Hg or cm H<sub>2</sub>O, above a set or reference point, which is the junction of the ventricle with the aorta. The question may be asked, what is the equivalent point in a fish immersed in a tank of water? In Figure 1 a simple manometer has been connected to the dorsal aorta of a fish. If the heart had ceased pumping, the pressure of the surrounding water, pressing on the tissues and the blood vessels in them, would force the blood up the manometer tube only as far as the surface of the water (Figure 1A). If, through the heart's action, a pressure of 10 mm Hg were developed (Figure 1B), the blood would rise up the tube by this amount: conversely (Figure 1C), if a vessel developing a sucking pressure of -10 mm Hg had been cannulated, the level in the manometer would be pulled below the water surface. This relationship remains valid regardless of the depth of the fish in the tank: when calibrating the blood pressure of a fish, the zero set point is the surface of the water. Flow through the blood vessels of fish in the ocean depths is not affected by the overall high pressure to which they are subjected. It is true that high pressure, like high temperature, increases the thermodynamic activity of physical and chemical systems; the hearts of deep sea fish, which cease beating at surface pressures, are restored to near-normal activity in pressure chambers at 80–120 atm (Pennec *et al.* 1988). The mechanism is not yet understood; perhaps pressure stabilizes the membranes of the cardiac muscle cells. But in blood vessels it is the relatively small differences in total energy along their length that effect flow through them, not the absolute pressure imposed by the water around the fish.

Figure 1. The measurement of blood pressure in fish; a vessel has been cannulated. In a dead fish *A*, the blood rises to the level of the water and the manometer registers zero pressure. In a live fish *B*, a positive pressure of 10 mm Hg is registered. In another vessel *C*, a sucking or aspiratory pressure of -10 mm Hg is present.



Terrestrial animals, and such air-breathing fish as emerge onto land, face the problem that blood has weight and increases the transmural pressure in the vessels of the dependent parts (Figure 2*A*). The greater transmural pressure in these vessels increases the volume of blood held in them. Brief periods of weightlessness increase the central venous pressure of man from 2.6 to 6.8 mm Hg (Norsk *et al.* 1987). Fish in water are spared this problem for they are surrounded by a liquid with a specific gravity very similar to that of blood, and the water pressure, (Figure 2*B, C*), acting through the tissues, exerts an equal and opposite pressure on the vessel wall. The vessel is thus not caused to dilate, as it would be in a land creature, and the delicate venous sinuses that surround, for example, the eye, do not change in volume as the fish changes from a head-up to a head-down position when feeding from the bottom. In an eel, held out of water by its jaw, the circulation fails because the blood in its central veins, lacking the support of the water, drains into the lower part of the body and does not reach up to the level of the heart.

Figure 2. The influence of gravity on blood vessel dilation on land and in water. *A*, A fluid-filled elastic tube in air. *B*, A similar tube immersed in water in a steel pipe. *C*, A fish feeding from the bottom; the numbers indicate units of pressure at increasing depths.





*Velocity and cross sectional area*

The flow of blood, measured in  $\text{ml min}^{-1}$ , issuing from the heart into the ventral aorta, is destined to pass in turn through the four segments of the circulation, the arteries, arterioles, capillaries and veins. In a 2 kg cod (*Gadus morhua*), this flow would be likely to be about  $35 \text{ ml min}^{-1}$ . If the fish is in a steady state this volume of blood must pass through each segment in a minute; it is termed the volume flow for the segment. In both the gills and the tissues, relatively few large arteries divide into more numerous small arteries and arterioles and very many capillaries; the total cross sectional area of the blood vessels of the segment increases greatly at this last division. It diminishes again, both in the vessels efferent to the gills, and in the great veins, as the many narrow vessels coalesce to form fewer, larger ones.

Velocity of flow through a segment is inversely proportional to its total cross sectional area; the 35 ml flowing each minute, through the gills or peripheral capillaries of the cod cited above, are shared amongst many thousands of channels and the blood flows very slowly indeed through any one. An analogy can be drawn with a mountain stream which includes, in its course down to the plains, two lakes, one near the summit and one half way down. The water flows slowly through the large cross section of the lakes but speeds up as it traverses the narrow gorges between. It is the cross sectional area of the whole segment that determines the velocity of flow through its vessels, not the calibre of the vessels that make it up. Hughes (1966) has calculated that the velocity of flow of the blood in the gill lamellae of the mackerel (*Scomber scombrus*) is  $0.036\text{--}0.0973 \text{ cm sec}^{-1}$ ; in the ventral aorta it would be likely to be several  $\text{cm sec}^{-1}$ . This low velocity provides time for the diffusional exchanges which occur in the gills and peripheral capillaries, to approach completion.

Flow through the circulatory system of a fish is effected by a variety of propulsive mechanisms. The heart of a fish is sometimes termed the branchial heart, to distinguish it from certain auxiliary hearts and propulsers. We will term it the heart and use adjectives such as caudal and portal to designate the minor ones. The heart is certainly the outstanding organ of the fish circulatory system and provides most of the energy necessary to make the blood flow. Its structure and function will be considered in Chapter 2.

## 2

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### THE HEART

#### Introduction

The heart is a pump composed largely of one particular kind of tissue, cardiac muscle. The hearts of fish (Figure 3A, B, C) consist of three or four chambers arranged in a single series. All fish have a sinus venosus into which the returning blood flows; it may have a layer of cardiac muscle, but in many teleosts (Figure 3C) this is reduced to a few scattered fibres and in others, muscle is lacking altogether. The atrium is a capacious, thinly-muscled sac which generates just sufficient pressure to fill the thickly-muscled ventricle. This is most commonly sac-like, but may be pyramidal, as in the Clupeidae, or tubular as in the hake (*Merluccius merluccius*) (Santer *et al.* 1983). It is the chief pressure-raising chamber of the heart and comprises 58–85% of its weight. The fourth chamber, in elasmobranchs and some primitive bony fish, is the conus arteriosus (Figure 3B); it is barrel-shaped and invested with contractile cardiac muscle. In most teleosts it is replaced by an elastic chamber, the bulbus arteriosus (Figure 3C), the wall of which contains much elastic tissue and some smooth muscle.

The efficient operation of such a heart depends on the sequential activation of its chambers. At the start some tissue must have the ability to generate a heart beat, for the fish heart, like that of other vertebrates, is myogenic. It is not activated by motor neurones like the heart of a crab. Furthermore, blood has mass and takes time to flow from one chamber to the next. Some mechanism to delay the forward march of excitation must be provided whilst flow occurs. The energy expended in accelerating the blood will increase as the square of the velocity of flow from one chamber to the next, and will ultimately be lost to the system as heat. A rapid acceleration and deceleration of blood from atrium to ventricle may be wasteful of energy.