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SHORT COMMUNICATIONS

BLOOD-FLOW VELOCITY IN MICROVESSELS OF THE GILL FILAMENTS OF THE GOLDFISH (*CARASSIUS AURATUS L.*)

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Many recent studies have been made on the nature of blood pathways through the gills of fish (Hughes & Grimstone, 1965; Vogel, Vogel & Kremers, 1973; Vogel, 1978; Laurent & Dunel, 1976), but as yet there have been no direct measurements of the blood flow velocity in any parts of this system. Estimates have been made of the circulation time through the gills (Mott, 1950), and this and other observations indicate that the blood remains in the gills several seconds. One of the reasons for this relatively slow circulation time is due to the large surface area (Gray, 1954; Hughes, 1966, 1972) of the secondary lamellae which form the sites for gaseous exchange between the water and blood. The network of blood channels which forms the microcirculation is, therefore, very extensive and consequently the velocity of blood flow in individual channels is relatively low.

Fish gill vessels are much more exposed than the pulmonary circulation and would seem more suitable for the application of some recent developments in instrumentation. Tanaka & Benedek (1975) measured blood flow velocity by means of a laser Doppler technique in which laser light was introduced into the venous blood stream through a glass fibre placed in the femoral vein of a rabbit. Stern *et al.* (1977, 1979) measured a flow parameter by means of a laser Doppler probe placed on human skin and exposed rat kidney.

Einav *et al.* (1975) measured the velocity profile in arterioles of hamster cheek pouches. Koyama, Mischina & Asakura (1975) measured the velocity profile in venules of frog web by means of a laser Doppler microscope (Mishina, Asakura & Nagai, 1974; Mishina, Koyama & Asakura, 1975). Horimoto *et al.* (1979*a*) studied the velocity profile in arterioles of the web of frog feet during the cardiac cycle. Using a similar technique, Koyama *et al.* (1979) and Horimoto *et al.* (1979*b*) measured the flow velocity in microvessels of lungs exposed on one side. These recent developments make it possible to measure flow velocity in microvessels, if they are accessible, without surgical invasion. It was therefore decided to try to apply this technique to the measurement of flow velocity in the gill microcirculation.

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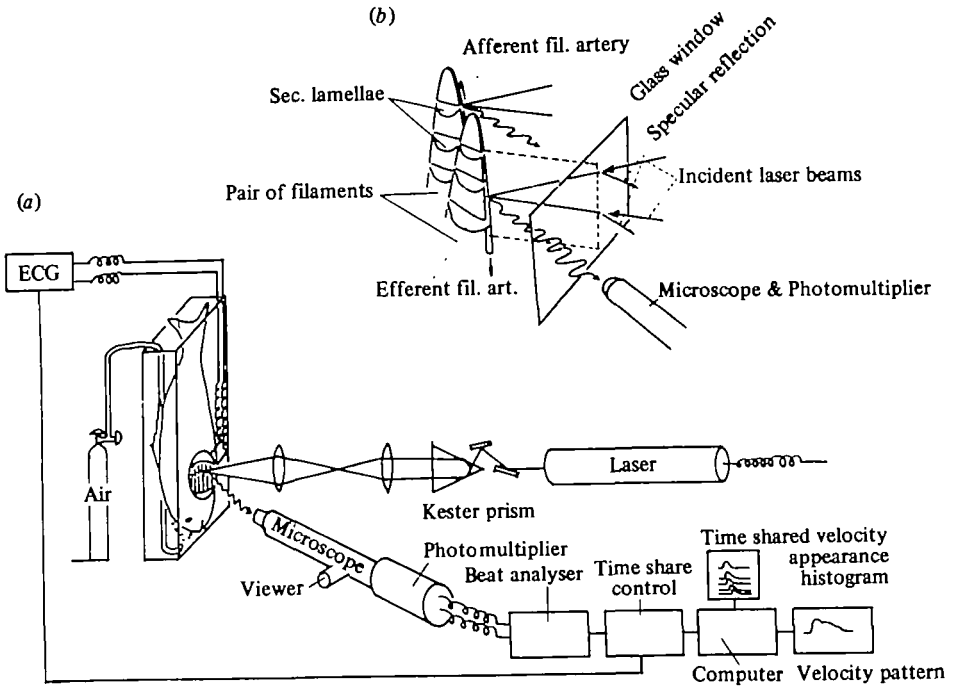

Fig. 1. (*a*) Schematic diagram of the laser–Doppler microscope as used to measure blood flow velocity in the gills of the goldfish. A laser beam is divided into two beams by means of a Kester prism. The dual beams are made to cross in a microvessel by manipulating the holder of the trough. The scattered laser light is collected by a microscope positioned in an oblique backward direction. Positioning of the beams and focusing is carried out by observation through the viewer of the microscope. The scattered light is detected by a photomultiplier coupled with the microscope. The output signal of the photomultiplier is transmitted to a beat analyser that measures the wave period of the output signal. The wave period of each output signal, which is inversely related to the erythrocyte flow velocity, is stored via a microcomputer in 16 flow-velocity-appearance frequency histograms, which are arranged for each 100 ms period of the cardiac cycle by a time-sharing control circuit triggered by the R-wave of ECG. Average value of each histogram is computed, the output giving the intermittent flow velocity pattern during a cardiac cycle.

(*b*) A ray diagram showing the incident and scattered light relative to the flow direction. The incident dual laser beams and the blood vessel are contained in one plane, while the scattered-light-collecting system should not have its optical axis in the same plane. Since specular reflexion takes place on the glass window apart from the probing area, only the scattered laser can be collected. The collection of the scattered light can be ascertained by the occurrence of a burst-like beat signal output from the photomultiplier. Blood flow in efferent filament arteries, of gill filaments splayed out from the first gill arch, can be clearly seen under a microscope. As the microscope is moved toward distal portions of the efferent filament artery, its diameter gradually decreases and at the tip, communication between the efferent and afferent arteries can be observed. A further movement of the microscope in the same direction reveals the tip of the paired filament in which the distal section of the afferent filament artery can be identified as the direction of blood flow is opposite to that of the efferent vessels. Thus, blood-flow velocity can be measured in afferent and efferent arteries of adjacent filaments. For the measurements in the marginal channel the trough was placed in an oblique position.

Goldfish (body weight 12–15 g) were obtained from a commercial supplier and were anaesthetized in a solution of MS 222 (0·125 mg/ml) and after removal of the operculum on one side were placed in a closely fitting trough which had a small window, through which the gills could be observed as they were held close to the

inner surface of the coverslip (Fig. 1). The trough was filled with water containing MS 222 (0·0125 mg/ml) and constantly aerated, but ventilation movements were absent during the recordings. Insulated-pin electrodes were inserted in front and behind the heart in order to record the ECG.

For the proper function of the laser Doppler microscope the dual beams must cross exactly at the point where the flow velocity is to be measured in the blood vessel being examined. However, an exact crossing of this kind was unexpectedly difficult in this preparation mainly because of the particular orientation of the secondary lamellae on the most accessible gill filaments. This problem was partly solved by placing the fish in a vertical position, as the orientation of the microscope was fixed (Fig. 1*a*, & *b*).

The microscope collected scattered laser receiving Doppler shifts by flowing erythrocytes and transmitted them to the photomultiplier (Koyama *et al.* 1976). The photomultiplier transformed the scattered light into electric currents, whose wave period was measured on-line by a beat signal analyser (Koyama *et al.* 1979; Horimoto *et al.* 1979*b*). The measured wave periods were processed by a time sharing control circuit triggered by the R-wave of the ECG and stored in a computer system. Finally a velocity appearance frequency histogram was automatically constructed for each time period after the R-wave. Then the average value of each histogram represents the statistically probable flow velocity for each time period of the cardiac cycle (Horimoto *et al.* 1979*b*). The velocity pattern was constructed by smoothly connecting the mean value of each histogram. The pulsation of flow velocity was expressed by the ratio of pulsatile amplitude (difference between the maximum and the minimum velocity during the cardiac cycle) against mean flow velocity (mean value of the area enclosed by the velocity pattern during the cardiac cycle). The whole experimental arrangement is shown schematically in Fig. 1*a*.

Measurements were carried out at a room temperature of 18–20 °C over 2 h periods and the fish recovered from such experiments within 1 h after their return to normal fresh water.

Blood flow through the efferent and afferent filament arteries was clearly visible with a microscope. The efferent artery is more accessible because of its position on the outer edge of each filament. As the microscope was moved towards the tip of a gill filament, the diameter of the artery gradually decreases and finally communicates at the tip with the afferent filament artery in which the blood flows in the opposite direction. Communication between afferent and efferent arteries takes place mainly through the large number of secondary lamellae, in each of which flow within the marginal channel was especially visible.

Examples of blood-flow velocity in the afferent and efferent filament arteries are shown in Fig. 2. In the afferent artery the flow velocity reached its maximum value 200 ms following the R-wave and thereafter decreased gradually. The velocity in the efferent filament artery was slightly less than that in the afferent artery and reached a maximum about 400 ms following the R-wave. The pulsatility was smaller in the efferent artery than in the afferent. Values for flow velocity and its pulsation in the afferent filament arteries of four goldfish averaged 0·65 ± 0·34 $mm.s^{-1}$ (S.D.) and 0·63 ± 0·22 for arteries whose diameter range from 12-15 μm respectively. Corresponding values for the efferent arteries having diameters ranging from 15-47 μm were 0·63

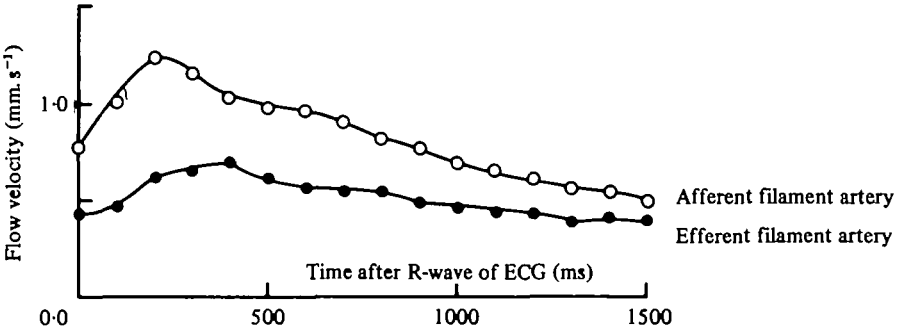

Fig. 2. Blood-flow velocity in the afferent and efferent filament arteries of a goldfish. In this particular experiment the difference in pulsatility was well marked, but the difference in velocity between the two arteries was greater than in all other preparations.

$\pm$ 0·34 mm.s^{-1} and 0·26 $\pm$ 0·20 respectively. The mean flow velocity in the marginal channel of a secondary lamella was 0·3 mm.s^{-1}.

The relatively large standard deviation in the flow velocities obtained from different fish is mainly attributable to variations in the depth of anaesthesia and perhaps the unnatural orientation of the fish. Flow velocities recorded were probably lower than those that occur under normal physiological conditions. The maximum flow velocity observed (i.e. about 1 mm.s^{-1} in both the afferent and efferent filament arteries) may represent the actual flow velocity. This velocity is smaller than that measured in the microvessels of frog lung (1·87–2·29 mm.s^{-1}) at a similar temperature. Blood-flow velocity in mammalian pulmonary microvessels has been determined only by means of a high-speed cinematographic technique. It is reported to be 1–2 mm.s^{-1} (Vogel 1947) and 0·7 mm.s^{-1} on average (Schlosser, Heyse & Bartels 1965). The blood-flow velocity in mammalian arteries and venules must be much larger than these values.

Pulsation in the velocity pattern is greater in the afferent filament artery than in the efferent artery, which is not unexpected in view of the extensive and compliant microvascular network within the secondary lamellae, which must have a smoothing effect.

In some preparations the differences in velocity measured in the afferent and efferent filament arteries was due to differences in the relative position at which the recordings were made and consequently differences in vessel diameter. In view of recent studies of blood pathways within gill filaments, it must be remembered that not all of the blood flowing in the afferent filament artery necessarily flows in the efferent filament artery, as some of it might enter the central venous sinus. Furthermore the dimensions of the afferent and efferent arteries are not equal at any given level of the gill filament.

The length of the secondary lamellae was measured in three goldfish. The approximate length of the marginal channel was obtained by multiplying width by $\frac{1}{2}\pi$. The results obtained indicate distances of 0·4 and 0·3 mm in the middle and tip regions of the gill filaments respectively. From these estimates and the measurements of flow velocity it is estimated that the contact time between erythrocytes in the secondary lamellae and the water is approximately 1 s, during which period oxygenation of haemoglobin must take place. In some previous experiments (Hughes & Koyama, 1974) it was found that the time for oxygenation across the tissue barrier

of isolated secondary lamellae was also approximately 1·0 s in the carp and eel. Estimates of blood flow velocity and contact time based upon secondary lamellar dimensions gave values of 0·36–0·73 mm.s^{-1} and 1·0–1·9 s respectively for the mackerel (Hughes, 1966).

It is concluded, therefore, that flow velocity in the filament arteries is slightly lower than 1 mm.s^{-1} at 18–20 °C and is more pulsatile in the afferent than in the efferent artery.

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