Occurrence of eddy flow in the flowing plasma space in capillary blood vessel of frog web

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Summary. Studies of capillary blood flow velocity by means of a laser Doppler microscope suggest the occurrence of eddy flow in the plasma space in the capillary blood vessel of frog web.

With assumption that the red blood cells are rigid particles filling the capillary lumen, it is theoretically considered that the eddies are produced in the plasma space between successive red blood cells flowing in the capillary vessel (Bolus flow model)[2,3]. However, the actual red blood cells are deformable in a capillary vessel whose diameter is smaller than 10 µm. In addition, actual measurements on the eddies in the functioning capillary vessel have not been reported, probably because there are no methods applicable to the measurements of localized flow in the capillary vessel of living animals. It remains still unknown whether the eddies really exist in the capillary of living animals. The purpose of the present study is to investigate the movements of particles whose diameter is smaller than that of red blood cells. The flow velocities of blood in the capillary vessel of frog web are measured by means of a laser Doppler microscope[4-6] with a photomultiplier having different sensitivities to obtain information about the eddy flow from the flow velocities of small particles.

In the laser Doppler microscope, 2 small beams split from 1 laser beam are crossed on a capillary vessel and the light scattered in that vessel with the Doppler shift is collected through a microscope onto the photomultiplier which is connected to the Doppler beat-signal analyzing system. The pedestal voltage of output signals from the photomultiplier is filtered out by means of a high-pass filter with the cutoff frequency of 200 Hz to yield Doppler beat signals of a clear burst type. A velocity-frequency histogram is automatically constructed in the signal analyzing system by summarizing the Doppler beat signals. The intensity of Doppler beat signals depends on the size of particles producing the Doppler shift for the incident light beams. With decreasing size of the particles, the burst signals become weaker. To detect the signals produced by the small particles, the sensitivity of the photomultiplier is increased by supplying its voltage to 1500 V in comparison with the supplied voltage of 1000 V in normal usuage.

For in vivo measurements, the frog (*Xenopus laevis* Daudin) is anesthetized by dipping in a 0.02% water solution of Ms 222® (Roche-Sankyo). Its web is stretched on thin gum plate and immersed in a water chamber to avoid desiccation. The velocity histograms obtained are normalized with the peak frequency and plotted in figure 1. Since the flow velocity of capillary blood varies spontaneously, the histograms show the broad distribution of flow velocities. However, it is well descernible that the histograms obtained with the supplied voltage of 1500 V shows a large deviation in the range of higher flow velocities up to 2 mm/sec, while the histogram obtained with that of 1000 V shows the upper limit of the distribution around the velocity of 1.5 mm/sec. To explain this phenomenon we may compare Doppler beat signals observable in the separated human blood plasma with those in the suspension of red blood cells in vitro. Histograms for the suspension of blood cells flowing through a glass capillary (100 µm in diameter) which are obtained with the 2 supplied voltages of 1000 and 1500 V show no difference in the distribution pattern of flow velocities. This suggests that the simple increase of the supplied voltage yields no changes on the velocity histograms. Examples of the beat signals are shown in figures 2 and 3. No beat signals are clearly recorded from the flowing plasma when the photomultiplier is charged at 1000 V (figure 2, A). When the supplied voltage is increased to 1500 V, the beat signals become observable from the same plasma flow (figure 2, B). When red blood cells are added to the plasma by the amount of 0.5 vol.%, the beat signals can be clearly observed even at the low supplied voltage of 1000 V (figure 3, A). 3 beat signals exceed the threshold of the signal-processing system as indicated by the white dotts recorded below, while other

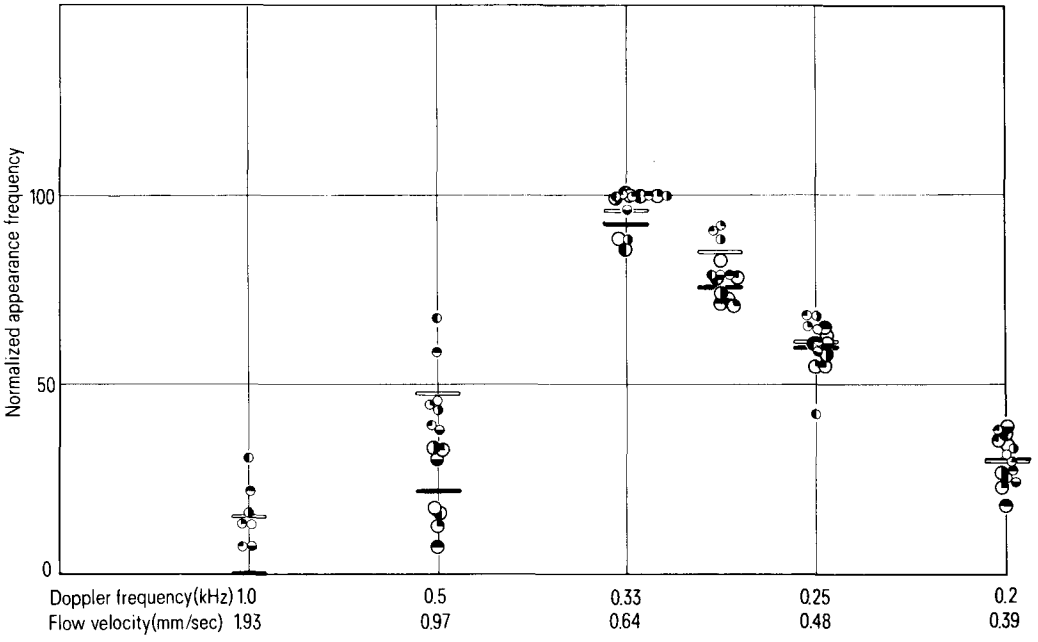

Fig. 1. Velocity-frequency histogram of the blood flow through a capillary vessel of frog web obtained from 7 serial measurements using the photomultiplier of high (applied voltage of 1500 V, denoted with small circles) and low (supplied voltage of 1000 V, denoted with large circles) sensitivities. Open and closed thick horizontal bars indicate the mean values observed with high and low sensitivities, respectively. Various small and large circles correspond to each of 7 measurements.

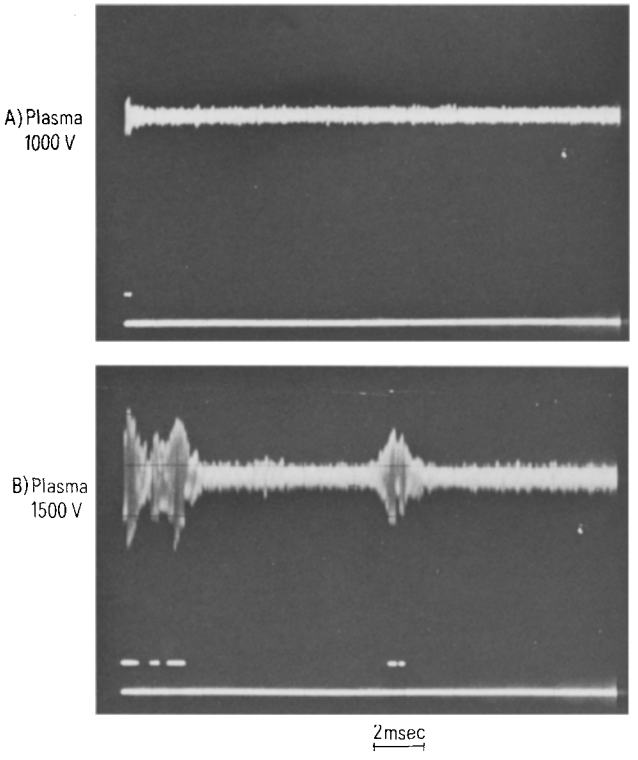

Fig. 2. Doppler beat signals obtained in the plasma flowing through a glass capillary. *A* 1000 V; *B* 1500 V.

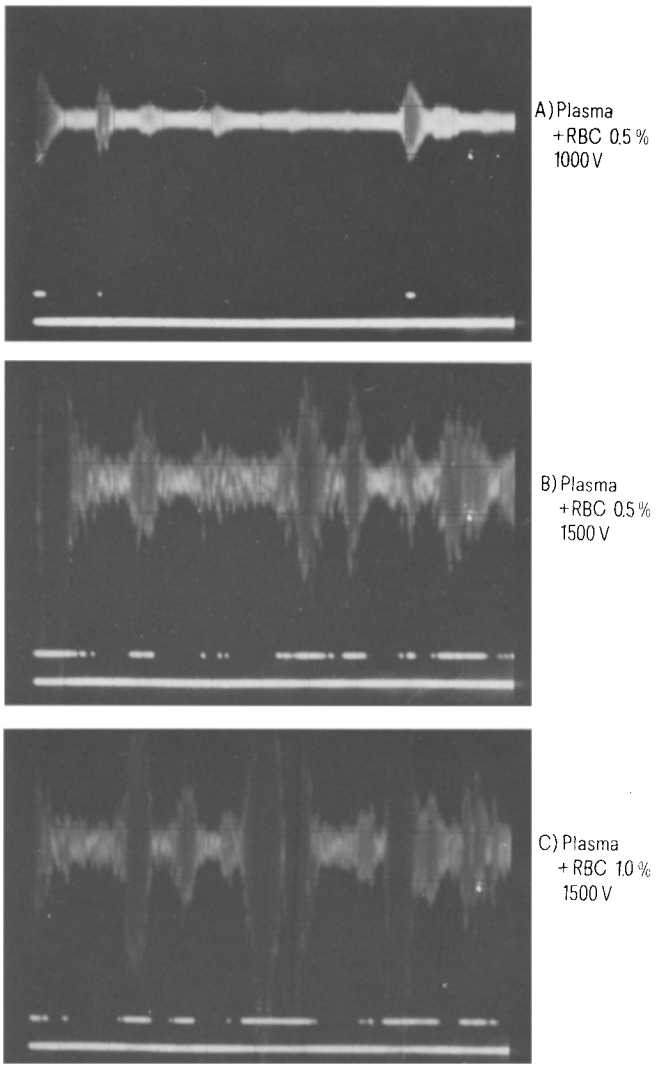

Fig. 3. Doppler beat signals obtained in the suspension of red blood cells flowing through a glass capillary. *A* 1000 V, suspension of red blood cells by 0.5 vol.%. *B* 1500 V, suspension of 0.5 vol.%. *C* 1500 V. The amount of red blood cells is increased to 1.0 vol.%.

5 beat signals are weaker than the threshold. By increasing the supplied voltage to 1500 V, the beat signals are markedly intensified. The 1st large beat signal is originated from a red cell and the 9 small beat signals recorded are weak ($\frac{1}{5}$ of the signal caused by red blood cells) but intensive enough to drive the signal-processing system (figure 3, B). When the amount of red cells is finally increased to 1.0 vol.%, the number of the strong signals is clearly increased (figure 3, C). The above results indicate that the elevation of photomultiplier sensitivity makes possible the detection of flow velocities due to some particles which are much smaller than the red blood cells. It may be concluded that a deviation of the histogram in figure 1 caused by the increase in photomultiplier sensitivity stems mainly from the participation of small particles probably contained in the frog blood. If some of the particles are assumed to flow more rapidly than red blood cells, the deviation in the range of higher flow velocities seems to be readily explained. When plasma is squeezed out through a thin capillary vessel and bordered by 2 successive red blood cells and eddies are consequently produced in the plasma space, the central portion of the plasma space containing the small particles flows more rapidly than the red blood cells. This interpretation, however, may raise the question why the histogram at 1500 V deviates only to the higher velocity. If the eddies are really present in the plasma space, the circumferential portion of the plasma space moves slower than the red blood cells, causing the broadening of the histogram also in the range of lower flow velocities. 2 reasons seem to be relevant for the observed histogram which deviated only to the higher flow velocity. (A) The pedestal voltage is filtered out by means of a high-pass filter at the lowest frequency of 200 Hz and; consequently the low flow velocities in the plasma eddies are filtered out by this process. (B) If the number of rapidly flowing particles is the same as that of slow particles, the rapidly flowing particles traverse more frequently through the probing field of the laser Doppler microscope than the slow particles. In other words, the laser Doppler microscope constructs a weighed histogram which is readily deviated to the range of higher flow velocities.

In conclusion, there obviously exist many particles which are smaller in size than the blood cells and flowing rapidly in the capillary vessel of frog web in comparison with the blood cells. The interpretation of this fact indicates that some flow eddies occur in the plasma space between successive red blood cells and move with the higher velocity compared to the red blood cells.

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